INFECTION BIOLOGY AND DYNAMICS OF INOCULUM PRODUCTION
BY CRONARTIUM QUERCUUM f. sp. FUSIFORME
ON QUERCUS RUBRA

BY

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Finally, deepest gratitude is given to my parents and children for their unending faith and encouragement without which this opportunity would not have been possible.

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The infection biology, role of environmental factors, and dynamics

of inoculum production by <u>Cronartium</u> <u>quercuum</u> (Berk.) Miyabe ex Shirai

f. sp. <u>fusiforme</u> (Cumm.) Burds. et Snow on northern red oak (<u>Quercus</u>

<u>rubra</u> L.) were investigated under laboratory conditions.

The scanning electron microscope was used to observe aeciospore

and urediospore wall morphology and the formation of germ tubes and

appressoria. Germination began within 15 minutes after aeciospores or

urediospores were placed on water agar or oak leaf discs. Aeciospores

and urediospores frequently produced branched germ tubes and appressoria

approximately 1 hour and 3 hours, respectively, after inoculation.

Directional growth toward stomata was not apparent. Appressorial forma-

tion and the production of infection hyphae occurred only over stomata.

The optimum temperature for aeciospore and urediospore germination

on water agar and leaf discs was 21 C and 18 C, respectively.

Appressorial formation from aeciospores and urediospores on leaf discs was maximum at 15 C and 18 C, respectively.

A minimum of 6 hours of oak leaf surface moisture was necessary for maximum aeciospore germination and appressorial formation. Appressoria were not formed when continuous leaf surface moisture was less than 3 hours duration. The percentage of aeciospores that formed appressoria increased with increasing duration of leaf surface moisture. Urediospore germination and appressorial formation increased steadily with increasing duration of surface moisture; maximum germination occurred after 24 hours of surface moisture. Leaf age did not influence aeciospore or urediospore germination or appressorial formation although interruption of the continuous moisture period delayed both events.

In vitro sporulation did not occur on leaf discs. The incubation period for the production of immature uredia on intact leaves was 6 days and 5 days following aeciospore and urediospore inoculations, respectively. Following aeciospore and urediospore inoculations the latent period for mature uredia was 7 days and 6 days, respectively.

The maximum infectious period for the population of uredial sori was 9 days following aeciospore inoculation of 2-day-old leaves. The maximum infectious period for the population of uredial sori was at least 24 days (which was the termination of the experiment) following urediospore inoculation of 2-, 5-, 7-, and 9-day-old leaves.

The incubation period for the production of immature telia was 8 days and 6 days following aeciospore and urediospore inoculations, respectively. Following aeciospore and urediospore inoculations the latent period for telia production was 9 days and 7 to 8 days, respectively.

Uredia preceded telia although uredial formation was not required for telial formation. Telia, but no uredia, were produced on leaves older than 6 days and 9 days when inoculated with aeciospores and uredio-spores, respectively. The average population ratios of telia to uredia for 2-, 5-, and 6-day-old leaves following aeciospore inoculation were 1.53, 2.00, and 31.00, respectively. The average population ratios of telia to uredia for 2-, 5-, 7-, and 9-day-old leaves following uredio-spore inoculation were 1.20, 3.82, 73.83, and 107.5, respectively. The maximum number of uredia and telia formed on leaves 5- and 6-day-old, respectively, when inoculated with aeciospores, and on leaves 2- and 7-days-old, respectively, when inoculated with urediospores. Secondary infection by urediospores was not evident after either aeciospore or urediospore inoculations.

Inoculum efficiency was calculated from inoculations with 1000 $\pm$ 100 aeciospores or urediospores per cm^2 on oak leaves. After 24 hours 34% of aeciospores germinated, 62% of these formed appressoria; .22 uredial sorus and 1.07 telial sori per cm^2 had formed after 20 days. After 24 hours 74% of the urediospores germinated, 28% of these formed appressoria; .66 uredial sorus and 2.63 telial sori per cm^2 had formed after 30 days.

INTRODUCTION

Fusiform rust, a destructive disease of slash and loblolly pines, limits effective management of these species and is of great economic importance in the South. Increases in distribution, incidence, and impact since 1930 have been significant and coincide with the increase in plantation management.

The causal fungus, Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme (Cumm.) Burds. et Snow, which attacks both the pine and the oak, has the potential to produce large quantities of inocula on both hosts. A significant positive correlation exists between fusiform rust incidence on pine and the abundance of susceptible oak and number of telia on oak. Therefore, research into the development of the epidemic on oak is important. Specifically, it is necessary to understand the infection biology and role of environmental factors in the dynamics of inoculum production by the pathogen on oak. The objectives of this study were: (i) to characterize the infection biology of C. quercuum f. sp. fusiforme on oak tissue in the laboratory; (ii) to determine the roles of temperature, leaf surface moisture, and leaf age on the production of inoculum by C. quercuum f. sp. fusiforme on oak leaves in the laboratory; and (iii) to quantify inoculum production from the germination of aeciospores through the occurrence of telia of C. quercuum f. sp. fusiforme on oak leaves in the laboratory in environmental conditions most favorable to this pathogen.

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These data will contribute to the subsequent development of a simulation model of inoculum production on oak.

LITERATURE REVIEW

Fusiform rust, caused by the macrocyclic, heteroecious basiodio-
mycete <u>Cronartium</u> <u>quercuum</u> f. sp. <u>fusiforme</u>, is increasing in forests
of the southern United States (Czabator, 1971). It is the most destruc-
tive disease of southern forests, and it significantly limits producti-
vity of slash pine (<u>Pinus</u> <u>elliottii</u> Engelm. var. <u>elliottii</u>) and loblolly
pine (<u>P</u>. <u>taeda</u> L.) throughout much of their ranges (Dinus and Schmidt,
1977).

Millions of dollars are lost annually because of damage to pines
by fusiform rust (Powers <u>et</u> <u>al</u>., 1974); an annual loss of 562 million
board feet of sawtimber and 194 million cubic feet of growing stock was
reported in this recent estimate. This is a yearly stumpage loss of
approximately 28 million dollars and a loss of 150 million dollars in
finished wood products. In 1974, 18.6 million cubic feet of wood and
3.9 million dollars were lost in Florida as a result of fusiform rust
(Phelps and Chellman, 1975). Rust incidence has increased as the inten-
sive management of southern pine forests increased (Schmidt <u>et</u> <u>al</u>.,
1974; Griggs and Schmidt, 1977).

Phelps (1977) evaluated fusiform rust incidence in slash pine plan-
tations in 10 southern states and reported increased incidence of the
disease and distribution of the pathogen relative to an evaluation made
35 years previously. Rust incidence is highest in a ridge extending
northeast to southeast through the central portions of South Carolina,

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Georgia, Alabama, and Mississippi. In Florida, fusiform rust incidence

increases from 0-15% in the south and along the east coast to 46-60% in

the northwest region of the state (Schmidt et al., 1974).

Twenty species and varieties of oaks are common natural primary

hosts of C. quercuum f. sp. fusiforme (Czabator, 1971; Dwinell, 1974).

In addition, 30 other species of Castanea, Castanopsis, Lithocarpus,

and Quercus have been inoculated successfully with aeciospores and

urediospores (Czabator, 1971). The black oak group, which includes

water oak (Q. nigra L.), willow oak (Q. phellos L.), southern red oak

(Q. falcata Michx. var. falcata), northern red oak (Q. rubra L.), and

turkey oak (Q. laevis Walt.), are much more susceptible to infection

than white oaks (Dwinell, 1974). Of the alternate hosts, loblolly and

slash pines are the two most susceptible species (Powers, 1975). Pond

pine (P. serotina Michx.) and longleaf pine (P. palustris Mill.) are

moderately resistant, while spruce pine (P. glabra Walt.), sand pine (P.

clausa (Chapm.) Vasey), Virginia pine (P. virginiana Mill.), and short-

leaf pine (P. echinata Mill.) are highly resistant (Goddard and Wells,

1977).

The pathogen, C. quercuum f. sp. fusiforme, is a basidiomycete in

the order Uredinales. The genus Cronartium is distinguished by

(i) sessile, one-celled teliospores united in columns; (ii) a prominent

aecial periderm; and (iii) pedicillate urediospores (Czabator, 1971).

Burdsall and Snow (1977) and Hedgcock and Siggers (1949) found that al-

though C. quercuum and C. fusiforme Cumm. attack different species of

pine, both have comparable life cycles, attack most of the same alter-

nate host species, and are morphologically identical.

Serological differences between C. fusiforme and C. querccum (Gooding and Powers, 1965), different pathogenic interactions on Quercus spp. (Dwinell, 1969), and significant differences in aeciospore sizes (Dwinell, 1969) have been demonstrated. However, these differences do not warrant separation into species (Burdsall and Snow, 1977).

The pathogenic differences of C. fusiforme and C. quercuum were investigated by Powers (1972) using aeciospore collections from different pine hosts. Isolates of C. quercuum from jack pine (P. banksiana Lamb.) produced 70% infection (trees with galls) on jack pine, but none on Virginia pine; the Virginia pine isolates produced galls on 43% of the Virginia pine seedlings and none on the jack pine. This demonstration of distinct physiological specialization was substantiated by Kais and Snow (1972). When pines of five species were inoculated uniformly with isolates of C. fusiforme and C. quercuum, differences in susceptibility of the pines were observed during the first year. Cronartium quercuum isolates were distinguished from C. fusiforme by gall shape and by host range. These results established that races of C. quercuum existed (Kais and Snow, 1972).

Burdsall and Snow (1977) suggested that the term formae speciales instead of races be used to designate the subspecies taxonomic groups of C. quercuum. Four formae speciales of C. quercuum were erected on the basis of their interactions on different pine species: C. quercuum f. sp. banksiana on jack pine, f. sp. virginiana on Virginia pine, f. sp. echinata, on shortleaf pine, and f. sp. fusiforme on loblolly and slash pine.

The life cycle of C. quercuum f. sp. fusiforme is initiated in the spring (March-June) when basidiospores (sporidia) from oak hosts are wind-disseminated and may infect pine. Germination and subsequent penetration by the spores occur on succulent hypocotyls, cotyledons, stem tissue, or primary needles (Miller, 1970). Penetration is either direct or through stomata (Miller et al., 1976; Patton and Johnson, 1966). Hyphae grow into stem and branch tissues as irregularly oblong primary hyphae, usually single-celled but occasionally several-septate (Miller et al., 1980). Unicellular, uninucleate, unbranched or occasionally branched haustoria ramify throughout perennial shoot tissues (Jewell et al., 1962). Host reaction to haustorial development is manifested by hypertrophy and hyperplasia and finally in the formation of typical spindle-shaped galls.

One or two years after infection, pycnia are produced in extensive, shallow cavities under the bark on the pine gall (Czabator, 1971). In the fall pycniospores are released in viscid, yellow to red drops (Hedgcock and Siggers, 1949). Their role is unknown, but in other rust fungi pycniospores function in diploidization (Walker, 1957).

Cronartium quercuum f. sp. fusiforme overwinters as mycelium in the vascular cambium and galls on the pine. Subsequently aecial sori are formed and produce nonrepeating, vegetative aeciospores, usually as a result of dikaryotization (Hiratsuka, 1973). The aecia are typically associated with pycnia (Hiratsuka, 1973). The aecial stage of many rusts forms within a few days following the appearance of pycnia (Little-field and Heath, 1979). Typically the aecia form on the abaxial surface of the leaf, although the sori may also appear on adaxial surfaces

(Littlefield and Heath, 1979). In coniferous hosts, aecia often occur in association with localized galls or swellings. These aecia typically do not appear until the season following the production of pycniospores, the latter not occurring until approximately 2 years after basidiospore infection (Boyce, 1961).

The aggregated hyphae of C. quercuum f. sp. fusiforme, which form the aecium, grow tangentially among host cells at a depth of 6 to 10 cells below the peridial layer (Colley, 1918). The hyphae force the cortical cells apart and masses of disassociated hyphae form between these cells which may extend to a depth of 15 to 20 cells in the cortex of the host (Jewell et al., 1962).

Rijkenberg and Truter (Littlefield and Heath, 1979) concluded that, in general, aeciospore initials are annellophoric in their mode of development, not meristematically arthrosporic as described by Hughes, 1970. An annulus remains on the wall of the aeciosporophore (Littlefield and Heath, 1979). After separation from the sporophore, while still in a chain, each aeciospore initial divides into an immature binucleate aeciospore (N+N) and a wedge-shaped intercalary cell (Littlefield and Heath, 1979; Hughes, 1970). The release of the mature aeciospore is aided by the disintegration of the intercalary or disjunctor cells (Littlefield and Heath, 1979). Hughes (1970) clearly showed that the ultimate cleaving of the aeciospores and intercalary cells is an arthrosporic process, while the formation of the aeciospore initials is an annellophoric process.

Each aecial sorus of C. quercuum f. sp. fusiforme produces large quantities of thick-walled orange spores that average 15.5 x 25.1 μm

(Czabator, 1971). The spores are obovate to ellipsoid, and coarsely verrucose but generally with a smooth area near the broad end of the spore (Czabator, 1971). Walkinshaw et al. (1967) found that the aecio-spores of C. quercuum f. sp. fusiforme had an extremely thick wall with annulated projections.

Aeciospores are formed and wind-disseminated in early spring (Hedgecock and Siggers, 1949). Aeciospore infection occurs only on young oak leaves (Czabator, 1971).

Walkinshaw et al. (1967) found aeciospore germination to be rapid, and, once initiated, the process continued in vitro until lysis of the elongated germ tube occurred. The average length of germ tubes produced on agar was approximately 320 μm, but lengths of 600 to 700 μm were common.

Typically the germ tubes from aeciospores form appressoria and penetrate their hosts by growing through stomata (Littlefield and Heath, 1979). Germination of the aeciospores results in a binucleate mycelium in the oak leaf tissue, from which uredia and telia are produced. The mycelium in the oak leaf is massed in the mesophyll, but ramifies into the palisade layer and under the upper epidermis, where the uredia and telia are formed (Czabator, 1971). Eleuterius (1968) suggested that typically only one uredium, followed by one or more telia, is produced by the mycelium derived from one aeciospore infection.

Uredia, in generaly, are sori which produce repeating, dikaryotic urediospores on a dikaryotic mycelium (Hiratsuka, 1973). Uredia form on leaves, stems, fruits, or fronds of herbaceous plants; however, none are reported to have formed on woody stems or branches as commonly happens with pycnia and aecia in perennial infections, as exemplified

by C. quercuum f. sp. fusiforme (Littlefield and Heath, 1979). Typical-
ly uredia are formed subepidermally, becoming erumpent; however, some
Hemileia spp. produce pedicellate urediospores that extend beyond the
leaf surface through stomata (Littlefield and Heath, 1979).

The fundamental cell in urediosporogenesis is the sporogenous cell;
each uredium contains many of these cells (Littlefield and Heath, 1979).
Elongated spore buds are produced sympodially through budding by the
sporogenous cells. Following conjugate nuclear division a septum forms
near the base of the elongated spore bud, separating the latter, which
has become the urediospore initial, from the sporogenous cell (Little-
field and Heath, 1979). Harder (1976) found a second conjugate septum
which separates the urediospore initial into a proximal pedicel and
distal, immature urediospore.

Urediospores of C. quercuum f. sp. fusiforme are ovate to obovate,
averaging approximately 14 x 21 μm in size (Czabator, 1971). Conical
spines that lack annulation cover the spore surface (Littlefield and
Heath, 1979).

Urediospores are wind-disseminated and are capable of reinfecting
the oak host (Czabator, 1971). Hedgecock and Siggers (1949) reported
that inoculating oak leaves with urediospores resulted in uredia in only
33% of the cases and that oak inoculation was not successful beyond the
second generation of urediospores in the greenhouse. However, Czabator
(1971) indicated that successive urediospore inoculation was successful
for several years at approximately 10-day intervals; this may not be an
important factor in the field (Czabator, 1971).

Germ tubes of urediospores usually form appressoria over a stoma
before infection occurs (Littlefield and Heath, 1979). Allen (1928)

found that two events were involved in germ tube growth and appressorial
formation: directional growth towards the stoma and induction of the
appressorium once the stoma has been encountered. Johnson (1934) first
postulated that directional growth of the germ tube may be a thigmotro-
phic response to the plant surface. He noted that the urediospore germ
tubes of _Puccinia graminis_ f. sp. _tritici_ Eriks. & P. Henn. grew pre-
dominantly along the transverse axis of the plant leaf. Maheshwari and
Hildebrandt (1967) also observed that _P. antirrhini_ Diet. & Holw. germ
tubes grew at right angles to the parallel ridges of the host cuticle,
and would do so even on isolated cuticles or leaf replicas.

Lewis and Day (1972) suggested that epicuticular wax is the primary
stimulus for the directional growth of _P. graminis_ f. sp. _tritici_ germ
tubes. However, some leaf surfaces lack this crystal lattice and direc-
tional growth still occurred (Maheshwari and Hildebrandt, 1967; Wynn,
1976). Thus the exact nature of the thigmotrophic stimulus of the leaf
surface is unknown and may differ among rusts. The observation of direc-
tional growth among germ tubes of various rust fungi suggests that a
thigmotrophic stimulus enhances the likelihood of stomatal penetration
(Littlefield and Heath, 1979).

Inoculation of oak leaves with _C. quercuum_ f. sp. _fusiforme_ aecio-
spores did not necessarily form uredia and often resulted directly in
the production of telia (Snow and Roncadori, 1965). These hair-like
telia form from the same mycelia that produce uredia (Eleuterius, 1968).
The telia are composed of hundreds of teliospores held together in a
matrix. Germination of the teliospores during moist periods produces
a promycelium bearing four uninucleate basidiospores (Hedgcock and

Siggers, 1949). Basidiospores are wind-disseminated and may infect pines.

Studies on variation in pathogenicity among C. quercuum f. sp. fusiforme isolates showed that the seedlings from one slash pine parent tree were more resistant to basidiospore inocula from Texas and Mississippi than to inocula from Alabama or Florida (Snow and Kais, 1970). Variability in pathogenicity among inocula was observed from different states, from various locations within a state and from different galls at one location (Snow et al., 1975). The largest difference in pathogenic variation occurred with inocula collected from the same individual gall. Snow et al. (1975) inoculated seedlings from open-pollinated slash pine families with known degrees of fusiform rust resistance with basidiospores of C. quercuum f. sp. fusiforme from diverse geographical sources. In one experiment, 10 pine families were exposed to inocula that had been collected along a transect across Texas, Louisiana, Mississippi, Georgia, and Florida. Two families were resistant to all inocula, three were uniformly susceptible, and the remaining families were resistant to some inocula. Virulence of isolates did not conform to any geographic pattern. Differential responses were detected in two families from approximately the same origin, which indicated extreme variability in the virulence of the isolates. An example of differential response was shown by pine Family six, which was resistant to inoculum from Louisiana, but susceptible to inoculum from Florida. Pine Family seven, by contrast, was more susceptible to inoculum from Louisiana than that from Texas. Pathogenic variability was observed among pine families from approximately the same geographical origin.

Favorable macroclimatic and microclimatic conditions are necessary for plant disease development. Each stage of the pathogen has particular requirements for development. The influence of temperature on the germination of aeciospores, urediospores, and teliospores of C. quercuum f. sp. fusiforme on water agar was studied by Siggers (1947). The following thermal limits of temperature on the germinating spores were determined: aeciospores, 11 to 29 C with an optimum at 21 C; uredio-spores, 8 to 29 C with an optimum at 17 C; and teliospores, 15 to 26 C with an optimum at 21 C. Reduced hyphal growth and apical curling of the germ tubes were observed commonly in tests in which the average temperatures ranged from 26 to 29 C.

The effect of environmental variation on the germination and subsequent appressorial formation in some rusts also has been studied. Vargas et al. (1967) found that urediospores of Puccinia cynodontis Lacroix germinated in a range of temperatures from 1.5 to 34 C with an optimum range of 18 to 34 C. However, light intensities greater than 200 ft-c during this period decreased germination. With P. coronata Cda. var. avenae Fraser & Led., Politowski and Browning (1975) found 21 C with 2 hours of dew period to be optimum for generation. Politowski and Browning (1974) also found that light significantly increased appressorial formation from the germinated aeciospore.

For some rusts the temperature at which the urediospores are produced influenced both the germination capacity of the spores and the response of the spores to storage (Czabator, 1971). With certain cereal rusts, urediospores formed at 16 C had better viability and germination than those which developed at 22 or 29 C (Hobbs, 1962).

In addition to temperature, moisture conditions are important variables in conditioning the incidence and distribution of rust diseases. Van Arsdel et al. (1956) found that the spread of white pine blister rust, caused by Cronartium ribicola Fischer, was limited principally by the lack of sufficient moisture for sporidial formation and germination. When periods of high relative humidity occurred, a prior period of 2 weeks without three consecutive days over 28 C became important to provide production of fertile teliospores.

Lack of precipitation may be a limiting factor for spread of fusiform rust in east Texas (Anon., 1977). Snow et al. (1968) found that infection of pine seedlings with basidiospores of C. quercuum f. sp. fusiforme was significantly related to the number of hours with relative humidity greater than 97%, and highest infection occurred on nights following afternoon or early evening rains.

High temperature in central Florida and low temperatures at the northern edge of the fusiform rust belt may be a limiting factor in disease development (Anon., 1977). Siggers (1949) reported that low temperatures in late winter retarded the development of oak leaves and decreased C. quercuum f. sp. fusiforme urediospore production.

Snow and Roncadori (1965) reported that uredial formation was significantly less on leaves 6 days old or older when inoculated with aeciospores. No uredia were formed on leaves 16 days old at the time of inoculation.

Measuring plant diseases and their determining factors is the domain of quantitative epidemiology which is concerned with the dynamics of the two interacting populations of host and pathogen (Kato, 1974; Kranz, 1974). Epidemics are complex and epidemiologists typically begin

their study at the single spore stage, i.e., the dispersal and infection unit. One infection unit may originate from one dispersal unit, but not all dispersal units are effective in establishing infection (Zadoks and Schein, 1979); effective dispersal units form only a fraction of those units produced.

Quantification of the number of dispersal units is imperative for a detailed understanding of the processes and subprocesses of an epidemic (Vanderplank, 1975). Vanderplank (1975) discussed the following relationships between the numbers of infections and amount of inoculum: (i) disease may be proportional to inoculum; (ii) disease may decrease in proportion to inoculum; (iii) an antagonistic interaction between spores may reduce the levels of effective inoculum and disease; or (iv) facultative synergism between spores may result in increased levels of effective inoculum and disease. As an example, Schein (Zadoks and Schein, 1979) found that approximately 50% of the urediospores of Puccinia recondita f. sp. tritici Rob. ex Desm. transferred to a leaf produced pustules. The author also found that the number of spores necessary to produce pustules in Uromyces appendiculatus (Pers.) Unger was approximately 11 to 1, or about 9% efficiency.

Infection efficiency is defined as the ratio between the number of spores applied per unit area and the resultant pustules formed per unit area (Zadoks and Schein, 1979). Infection efficiency may ultimately be viewed in the form of life tables which contain such vital statistics as the numerical composition of a population, its age distribution, the survival ratios for various age classes, and each group's life expectancy (Zadoks and Schein, 1979). Cohort life tables, in which a group of individuals of the same age from birth to death is studied, do not

depend on age classification and may be used to study pathogen populations (Zadoks and Schein, 1979). For example, cohort life tables for P. recondita isolate 1037 on primary leaves of wheat have been developed and the survivorship relations between parasite and host may be used effectively to compare interactions among races and cultivars (Zadoks and Schein, 1979).

Spore survivorship relations may also be studied to determine the effect of environment on components of the life tables. Environmental effects on infection processes, and ultimately on infection efficiency, may be designated as the stimulus–response relation which may evoke a definite and unique response relationship in either a qualitative (all-or-none response) or in a quantitative manner (percentage of spores which germinated, etc.) (Zadoks and Schein, 1979).

Simulation of a plant disease attempts to replicate the essence of this interactive system, i.e., host, parasite, and climate over time (Teng and Zadoks, 1980). Quantification of infection processes is an integral component of many disease simulation models. Many such models have been developed: Waggoner and Horsfall (1969) with tomato early blight – EPIDEM, Zadoks (1965) with wheat rust, Berger (1976) and Berger (unpublished data) with Cercospora leaf spot, and many others. Simulation models for fusiform rust have not been developed because the necessary information is not available and this is particularly true for the development of the epidemic on oak.

MATERIALS AND METHODS

Microscopic Examination of Infection Biology

Northern red oak acorns were obtained from the Bent Creek Fusiform Rust Testing Center of the U. S. Forest Service Southeastern Forest Experiment Station located near Asheville, NC. Acorns were germinated in the greenhouse in flats containing a sand-vermiculite mixture and were transplanted to 6-inch pots containing sand-vermiculite mixture. The seedlings were maintained in a shade house and watered twice a week without fertilization prior to inoculation with aeciospores or urediospores.

Aeciospores of C. quercuum f. sp. fusiforme were obtained in March, 1977-80 from galls occurring on slash pines near Valdosta and Macon, Georgia, and Gainesville, Florida. The spores were collected using hand-suction bottles; collections from individual areas were mixed. The spores were vacuum-dried in a desiccator at room temperature.

The aeciospores were stored in sterile vials at 4 C for 1 year. Prior to inoculation the spores were hydrated for 24 hours at 25 C in 100% relative humidity in a 10 mm deep petri plate. Prior to their use in experiments, a suspension of aeciospores in distilled water was sprayed onto water agar plates; after 6 hours viability was assessed via percentage germination. A spore was considered to be germinated when the germ tube equalled the shortest diameter of the spore. Only

spores from those vials in which 25% to 75% of the spores germinated were used in subsequent experiments. No attempt was made to separate spores from individual galls on locations or to identify sources of spores for individual experiments.

The adaxial surfaces of 2- to 8-day-old oak leaves were sprayed with an aqueous suspension of aeciospores (approximately 250,000 $\pm$ 25,000 spores/ml). The concentration was determined using a hemacyto- meter and was used in all subsequent aeciospore inoculation experiments. Inoculated trees were covered with opaque plastic bags for 24 hours to maintain high relative humidity. The bags were removed and the seedlings exposed to low light intensities in a 12-hour alternating light-dark schedule until uredial pustules formed. Throughout this incubation period, the seedlings were maintained in a growth chamber at 21 C.

Uredia developed on the abaxial surface of leaves of northern red oak seedlings previously inoculated with the aeciospores. Urediospores were collected with a small camel's-hair brush and transferred to petri plates lined on the bottom with filter paper. The plates were covered and stored at 4 C for approximately 24 hr prior to inoculations. Prior to their use in experiments, the urediospores were applied with a camel's-hair brush onto a water agar plate and evaluated after 6 hours for germination. Only spores in which 25% to 79% germination occurred were used in the experiments.

Two- to eight-day-old oak leaves were mechanically inoculated with an aqueous suspension of urediospores (approximately 250,000 $\pm$ 25,000 spores/ml) using a camel's-hair brush. This concentration of spores was determined using a hemacytometer and was used in all subsequent

urediospore inoculation experiments. Inoculated trees were covered with plastic bags for 24 hours to maintain high relative humidity. The bags were removed and the seedlings exposed to a 12-hour alternating light-dark schedule in the growth chamber until uredial pustules formed.

Suspensions of aeciospores or urediospores were sprayed onto northern red oak leaf discs that were randomly excised from the leaves. Three leafdiscs, each approximately 0.4 cm in diameter, per plate were sprayed with each suspension resulting in a density of 1000 ± 100 spores per cm^2 of leaf disc surface. This density was determined by ten sample counts of spores/cm^2: aeciospores - 947, 1010, 1101, 1074, 1090, 982, 1019, 927, 992, and 1073; urediospores - 951, 1009, 1076, 1053, 1006, 962, 1065, 926, 919, and 1042. These plates were incubated in an incubator at 21 C (aeciospores) and 18 C (urediospores) for up to 24 hours. Water agar plates without leaf discs were similarly sprayed with spore suspensions and incubated for determination of spore viability.

Aeciospore and urediospore germination and appressorial formation were examined using the electron microscope. Leaf disc samples inoculated with either aeciospores and urediospores were fixed at room temperature for 1 hour in a mixture of 2% gluteraldehyde and 2% para-formaldehyde in 0.025 M cacodylate buffer adjusted to pH 7.2 (Benny and Aldrich, 1975). Specimens were dehydrated progressively in a graded ethanol-distilled water series of 5, 10, 50, 75, 95, and 100% ethanol concentrations. Each leaf disc specimen was dehydrated for 5 minutes in each of the first five dilutions and for 30 minutes in the final concentration. The discs were critically point-dried using liquid carbon dioxide in a Model H Critical Point Dryer. Each leaf discs was coated, using an IB^2 Ion Coater

with a thin layer of gold under vacuum for 6 minutes. Specimens were observed using a S-450 Scanning Electron Microscope (SEM).

Additional aeciospores and urediospores were observed for spore shape and surface ornamentation without fixation, dehydration or critical point-drying. Aeciospores and urediospores were brushed onto cellophane tape placed on SEM stubs and coated with a thin layer of gold under vacuum for 6 minutes prior to viewing.

Influence of Environmental Factors

Temperature Experiments -- Aeciospore Inoculations

The effect of temperature on spore germination and appressorial formation of _C. quercuum_ f. sp. _fusiforme_ was investigated. Ninety-one water agar plates were inoculated with aeciospores and incubated in the dark for 24 hours at each of seven temperatures (10, 15, 20, 21, 25, 27, and 30 C). In these and subsequent experiments, temperature was observed to vary approximately $\pm$ .5 C. One plate was examined hourly for 1 to 12 hours and then 24 hours after inoculation using a reflected light microscope at 200X. Percentage germination of 100 spores in each of three replications was recorded. The optimum temperature for germination was determined to be 21 C. This temperature was used in subsequent experiments to assess aeciospore viability.

In a similar manner ninety-one water agar plates each with three oak leaf discs were sprayed with aeciospores and incubated. One plate was examined hourly for 1 to 12 hours and then 24 hours after inoculation at each temperature, and each leaf disc was observed using the reflected light microscope at 200X. One hundred spores of each of three

discs per plate were observed and percent germination was recorded. The percentage of spores which formed appressoria (number of spores which produced appressoria divided by the number of spores which germinated) was also recorded.

In an attempt to describe the nature of the associations between percent germination and time, and percent appressorial formation and time, various linear and non-linear models were examined. A three parameter logistic equation was used to describe these data for the different temperature (and leaf age) experiments. It is recognized that there may exist alternative models which describe these data as well, if not better than, the logistic model. The logistic curve was chosen because it has been shown to provide acceptable empirical description of data of this type in biology. Recent work (Zadoks, 1978) has suggested that other models which do not have the symmetric sigmoidal shape requirement of the logistic curve may be more appropriate, but are much more complex to apply and interpret. The logistic equation used in this study is:

$$Y = \frac{D}{1 + e^{a-bt}} \tag{1}$$

where, Y = germination or appressorial formation (%)

D = theoretical maximum limit of germination or appressorial formation (%)

b = initial relative rate (% per hour)

a = location parameter (such that $\frac{a}{b}$ has unit time)

t = time (hours)

e = exponential constant (2.7183...).

Non-linear regression was used to obtain the parameter estimates which resulted in best fit.

To give some indication of goodness of fit of the logistic curve to these data, a measure of percentage variation (R^2) explained by the model was computed for each fit. This measure is given as

$$R^2 = [1 - \frac{MSE}{MSTC}] \tag{2}$$

where,

R^2 = proportion of variation explained,

MSE = mean squared error from the fitted model,

MSTC = mean squared total corrected for the mean.

The times to 10% and 90% maximum germination and appressorial formation were calculated in an attempt to provide useful epidemiological parameters of percent germination or percent appressorial formation. The following equations were used:

Time to 10% maximum ($t_{.10}$),

$$0.10 \; D = \frac{D}{1 + e^{a-bt}} \tag{3}$$

$$t_{.10} = \frac{2.21 - a}{-b} \tag{4}$$

Time to 90% maximum ($t_{.90}$),

$$0.90 \; D = \frac{D}{1 + e^{a-bt}} \tag{5}$$

$$t_{.90} = \frac{1.97 + a}{b} \; . \tag{6}$$

The maximum rate (percent per hour) of germination or appressorial formation is the maximum of the derivation of y with respect to t, given as

$$\frac{dY(t)}{dt} = \frac{Dbe^{a-bt}}{(1 + e^{a-bt})^2} \; . \tag{7}$$

By setting to zero the second derivative of Y with respect to t, which
is

$$\frac{d^2 Y(t)}{dt^2} = \frac{Db^2 e^{a-bt}}{(1 + e^{a-bt})^3} (1 - e^{a-bt}) \quad , \tag{8}$$

and solving for t, the time (hours) at which the maximum rate occurs
is given as

$$t_{max} = \frac{a}{b} \quad . \tag{9}$$

This is, in fact, the time to 50% maximum theoretical germation or ap-
pressorial formation. Given the estimates of t_{max} and b an estimate of
a can be obtained by use of equation (9) above. The maximum rate is
then given by evaluating $\frac{dY(t)}{dt}$ at the value t_{max}. This is given as

$$r_{max} = \frac{dY(t)}{dt} \bigg| t=t_{max} = \frac{Db}{4} \quad . \tag{10}$$

The quantities t_{max} and r_{max} were computed to provide additional descrip-
tion of the data.

Similar analyses were conducted for germination and appressorial
formation data obtained from all temperature and leaf age experiments
using both aeciospore and urediospore inoculations.

Temperature Experiments -- Urediospore Inoculation

Seventy-eight water agar plates were inoculated with C. quercuum
f. sp. fusiforme urediospores and incubated in the dark for 24 hours
at each of six temperatures (10, 15, 18, 21, 25, and 30 C). One plate
was examined hourly 1 to 12 and then 24 hours after inoculation using a
reflected light microscope to determine the percent germination and ap-
pressorial formation for three replications of 100 spores. The optimum
temperature for germination was 18 C. This temperature was used in sub-
sequent experiments to assess urediospore viability.

In a similar manner seventy-eight water agar plates each with three leaf discs were sprayed with urediospores and incubated. One plate was examined hourly 1 to 12 and then 24 hours after inoculation at each temperature and each leaf disc was observed with a reflected light microscope at 200X. One hundred spores on each of three discs per plate were observed and percent germination and appressorial formation were recorded.

Leaf Surface Moisture Experiments -- Aeciospore Inoculations

For studies on the effect of leaf surface moisture on spore germination and appressorial formation by C. quercuum f. sp. fusiforme, two incubation humidity levels, wet and dry, were established to control leaf surface moisture. The wet level was developed by covering plates of water agar with leaf discs which had been inoculated with an aeciospore suspension. The dry level was developed by uncovering plates of water agar with leaf discs similarly inoculated. A container of silica gel was placed in the dry-level incubator to reduce relative humidity which removed the free surface moisture necessary for spore germination.

Ninety-four plates of water agar with leaf discs were sprayed with an aeciospore suspension and exposed for each of 10 moisture periods (0 to 9 hours) of consecutive wetness at 21 C. For example, 13 plates with inoculated leaf disc were incubated without lids (0 hour wet); 13 plates with inoculated leaf discs were incubated in the wet incubator for one hour and transferred to the dry incubator for the remainder of the experiment (23 hours); 12 plates with inoculated leaf discs were incubated in the wet chamber for 2 hours and transferred to the dry incubator for the remainder of the experiment (22 hours), etc. At the

end of the experiment leaf discs were viewed microscopically to deter-
mine percentages of aeciospore germination and appressorial formation.

The relationships between percent germination or percent appressor-
ial formation and leaf surface moisture with both aeciospore and uredio-
spore inoculations were examined using polynomial regression analysis.
The intent was to determine whether linear or non-linear (quadratic)
trends were evident in the data.

The effect of interruption of the free moisture period on percent-
age aeciospore germination and appressorial formation was studied. In
one experiment, eight plates of water agar with leaf discs were inocu-
lated with an aeciospore suspension and place in the wet incubator for
2 hours. No significant differences in germination or appressorial
formation after at least 6 hours of continuous moisture had been formed
in previous experiments. Thus, interruption of moisture periods of less
than 6 hours was investigated. At the end of both 1 and 2 hours in the
wet incubator, one plate was observed microscopically for percent spore
germination and appressorial formation. The remaining six plates were
transferred to the dry incubator for 6 consecutive hours. At the end of
this period one plate was observed for percent spore germination and
appressorial formation. The remaining five plates were returned to the
wet incubator. Four of these five plates were examined hourly and
the fifth plate was observed after 24 hours; percentage germination and
appressorial formation were recorded.

In another experiment on the interruptions in the leaf surface
moisture, eight plates of water agar with leaf discs were inoculated and
placed in the wet incubator for 6 hours and then transferred to the dry

incubator for two hours. At the end of the period, the plates were returned to the wet incubator for 16 hours. Individual plates with the three leaf discs were observed hourly from 6 to 12 hours and again at 24 hours; percentage spore germination and appressorial formation were recorded.

Leaf Surface Moisture Experiments -- Urediospore Inoculations

The effect of continuous moisture of C. quercuum f. sp. fusiforme urediospore germination and appressorial formation was investigated. Thirteen plates of water agar with leaf discs were inoculated with a urediospore suspension using a camel's-hair brush and exposed to 0 to 6 and 24 hours of consecutive wetness of 18 C. The plates were transferred between the wet and dry incubators over this period in a similar manner as indicated in the aeciospore moisture experiment. Evaluations of the number of urediospores that germinated or formed appressoria were conducted using polynomial regression analysis as discussed in the aeciospore moisture experiment.

The effect of an interruption of the free moisture period was studied to determine the influence upon urediospore germination and appressorial formation. In one experiment, nine plates of water agar with leaf discs were inoculated with a urediospore suspension and placed in the wet incubator for one hour before transfer to the dry incubator for one hour. One plate was evaluated hourly for percent spore germination and appressorial formation. The remaining plates were returned to the wet incubator and observed hourly from 3 to 8 hours and again at 24 hours; percentage germination and appressorial formation were recorded.

In the second experiment, the plates of water agar with leaf discs were placed in the wet incubator for one hour, transferred to the dry incubator for three hours, and returned to the wet incubator for the remaining 20 hours of the experiment. The plates were evaluated hourly for percent germination and appressorial formation from 2 to 9 hours and again at 24 hours after inoculation.

Leaf Age Experiments -- Aeciospore Inoculations

The effect of oak leaf age on C. quercuum f. sp. fusiforme spore germination and appressorial formation was the final environmental variable studied. Three discs were each removed from northern red oak leaves which were 2, 4, 5, 6, 11, and 16 days old. The discs from each leaf-age category were placed onto one petri plate containing 2% water agar and sprayed with an aeciospore suspension. The 11 plates were covered to maintain high leaf surface moisture and placed in the dark at 21 C for 24 hours. Aeciospore germination and appressorial formation were recorded.

Leaf Age Experiments -- Urediospore Inoculations

Three discs were removed from northern red oak leaves which were 2, 4, 9, 13, and 20 days old to study the effect of leaf age on urediospore germination and appressorial formation. The three leaf discs from each leaf-age category were placed onto one 2% water agar plate and inoculated using a camel's-hair brush with an urediospore suspension. The 11 plates were covered to maintain free moisture on the leaf surface and placed in the dark for 24 hours at 18 C. Urediospore germination and appressorial formation were recorded.

Quantification of Inoculum

To quantify uredial and telial inoculum production in the labora-
tory by C. quercuum f. sp. fusiforme, oak seedlings were inoculated with
aeciospores or urediospores. The seedlings were maintained in a growth
chamber at 21 C with low levels of light with a 12-hour alternating
light-dark schedule.

One northern red oak seedling from each of five foliar age classes
(2, 5, 6, 8, and 11 days since initial flush) was inoculated with an
aeciospore suspension providing 1000 ± 100 spores/cm^2 on the leaf sur-
faces. These trees and an uninoculated control were covered with large
opaque plastic bags to maintain high relative humidity and placed in a
growth chamber at 21 C. After 24 hours, the bags were removed and the
fungus allowed to incubate for an additional 21 days in a 12-hour, al-
ternating light-dark schedule. Immature uredia and telia were dis-
tinguished by the presence of non-spore producing sori which resembled
conical frustrums. An incubation period was defined as the number of
days between inoculation and the appearance of the immature sori. The
latent period was defined as the number of days between inoculation
and the appearance of mature (or spore-producing) sori. The cumulative
daily number of immature and mature uredia and telia per leaf age
class was recorded.

One northern red oak seedling from each of five foliar age classes
(2, 5, 7, 9, and 11 days since initial flush) was inoculated using a
camel's-hair brush with a urediospore suspension providing approximately
1000 ± 100 spores/cm^2 on the leaf surfaces. These trees and an uninocu-
lated control were covered with large opaque plastics bags to maintain

high relative humidity and placed in a growth chamber at 21 C. After 24 hours, the bags were removed and the fungus allowed to incubate for an additional 29 days at a 12-hour alternating light-dark schedule. The cumulative daily number of immature and mature uredia and telia per leaf age class was recorded. The average number of uredial or telial sori formed per age class was averaged for all leaf age classes that exhibited these sori.

Insufficient data were available to model the relationship between leaf age and average number of uredia and telia produced from either aeciospore or urediospore inoculations; therefore, simple means and standard error were calculated. To provide an indication of significance between these means, t-tests were performed at the $\alpha = .05$ level. Due to the small number of observations, the conclusions of these tests are tentative.

Inoculum efficiency tables were calculated for the average number of uredial and telial sori formed within all leaf age classes after inoculation with either aeciospores or urediospores. The time periods of infection and disease development with aeciospore and urediospore inoculations at optimal temperature and moisture conditions on Q. rubra seedlings were determined.

RESULTS

Microscopic Examination of Infection Biology

The orange, oval aeciospores from field collections of C. quercuum f. sp. fusiforme exhibited smooth and verrucose areas separated by transitional zones (Fig. 1-A). The spores had free standing, well-defined, and elongated verrucae (surface projections) with distinct annulations (Fig. 1-B).

The appearance of a germ tube began within 15 minutes after the aeciospores were sprayed onto all age classes of leaf discs or onto water agar. Germinated aeciospores produced branched and unbranched germ tubes (Fig. 2-C, D). Growth of germ tubes towards specific stomata appeared random. Bright orange, bulbous appressoria formed only over stomata in approximately four hours after inoculation onto all age classes of leaf discs (Fig. 2-D, E). Appressoria formed from aeciospores that exhibited branched and unbranched germ tubes. Infection hyphae were observed in the stomatal chamber (Fig. 2-D). Penetration occurred only through stomata.

The latent period for production of immature and mature uredia from aeciospore inoculation was 5-6 days on northern red oak leaf discs. Erumpent uredial pustules appeared on the abaxial leaf surface and the echinulate urediospores exhibited no surficial annulations (Fig. 3-F, G, H).

Within approximately 15 minutes after inoculation with uredio-
spores, branched and unbranched germ tubes were produced on leaves of
all age classes and on water agar. Bright orange, bulbous appressoria
formed only over stomata (Fig. 3-1). Penetration occurred only through
stomata.

The incubation period for the production of immature telia was
8-9 days (Fig. 4-J). The latent period for the production of mature
telia was 8-11 days (Fig. 4-K).

Influence of Environmental Factors

Temperature Experiments -- Aeciospore Inoculations

The optimum temperature for germination of aeciospores on water
agar was 21 C (Fig. 5). Approximately 76% of the spores germinated after
24 hours at this temperature. At temperatures above and below this
level, percentage germination decreased. For example, approximately
10% and 1% of the spores germinated at 10 and 30 C, respectively. Per-
centage germination was higher at temperatures approaching the optima;
approximately 60% and 70% of the spores germinated at 15 and 20 C,
respectively.

The effect of temperature on parameters of aeciospore germination
on water agar was analyzed using non-linear regression. The theoreti-
cal maximum (D) for percent spore germination was 76% and the maximum
germination rate occurred after 3.5 hours at this temperature. An
additional 1.4 hours (4.9 hours total) were required to attain 90% of
the theoretical maximum germination. At 15 and 20 C, respectively, pro-
gressively greater theoretical maxima for spore germination were

Figure 1 - (A,B). Electron photomicrographs of aeciospores of
         Cronartium _quercuum_ f. sp. _fusiforme_: A) Aeciospore
         exhibiting smooth and verrocose areas (6240 X; bar =
         6.7 μm); B) Aeciospore exhibiting annulated spore
         wall projections (a) (11325 X; bar = 6.7 μm).

A

B

a
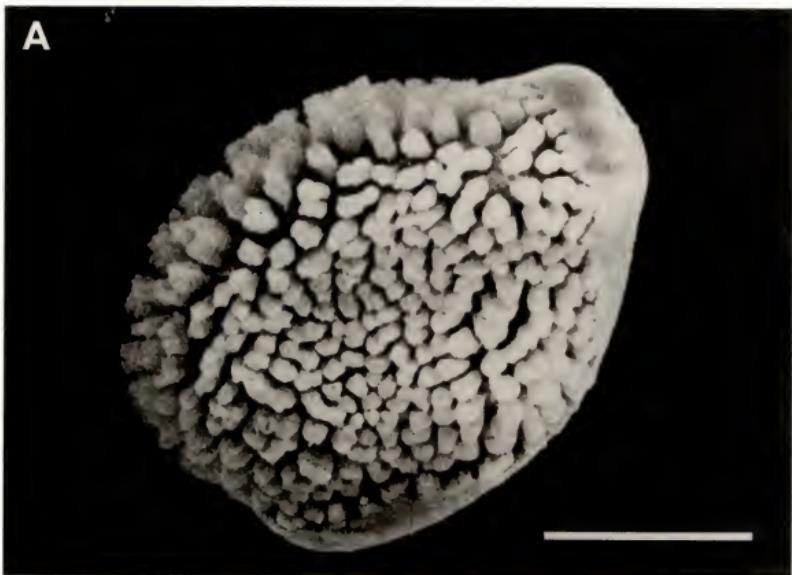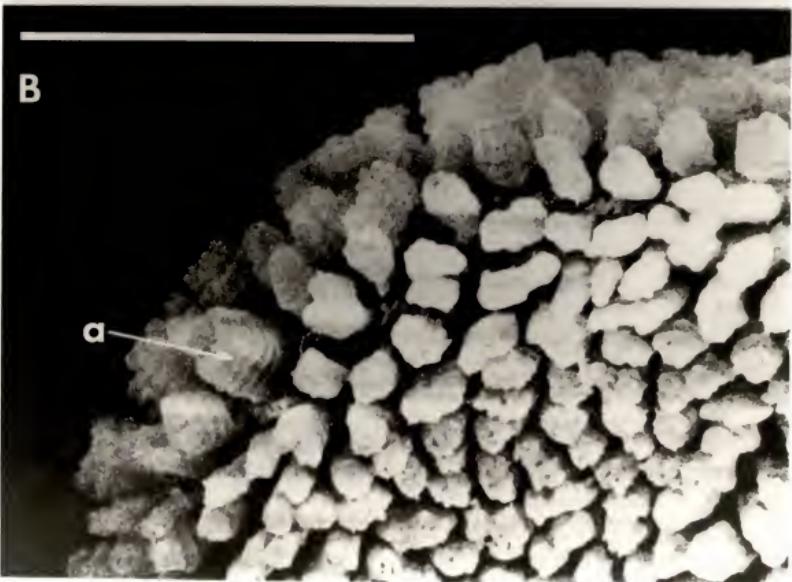
Figure 2 – (C,D,E). Electron photomicrographs of germination follow-
ing inoculation with aeciospores of Cronartium quercuum
f. sp. fusiforme on Quercus rubra leaf tissue: C) Branched
germ tube (g) of aeciospore (s) on abaxial surface of leaf
disc (2813 X; bar = 5μm); D) Germinated aeciospore (s)
with germ tube (g) and appressorium (a) formed over stoma
(1350 X; bar = 4.5 μm); E) Enlarged portion of appres-
sorium (a) with infection hyphae (p) (2511 X; bar = 5 μm).
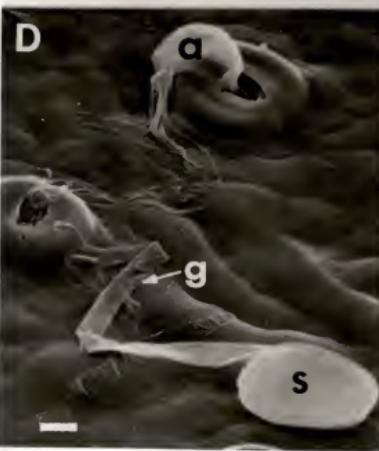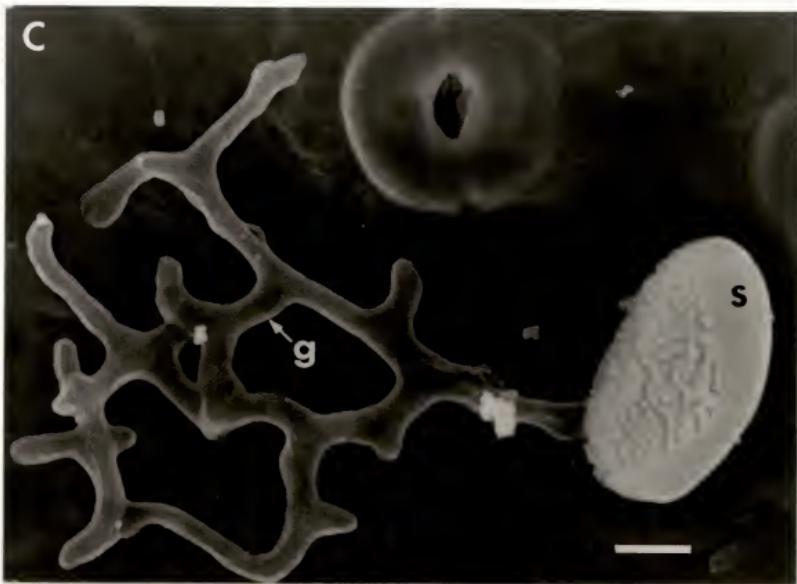
Figure 3 - (F,G,H,I). Electron photomicrographs of uredial pustules
        and urediospores of <u>Cronartium quercuum</u> f. sp. <u>fusiforme</u>
        on <u>Quercus rubra</u> leaf tissue: F) Erumpent uredial pustule
        with urediospores (220 X; bar = 50 μm); G) Uredial pustule
        with echinulated urediospores (1170 X; bar = 6.7 μm);
        H) Ovate urediospore (6417 X; bar = 6.7 μm); I) Uredio-
        spore (s) with branched germination tube (g) and appressorium
        (a) formed over stoma (1300 X; bar = 5 μm).

F

G

H

I

s

g

a
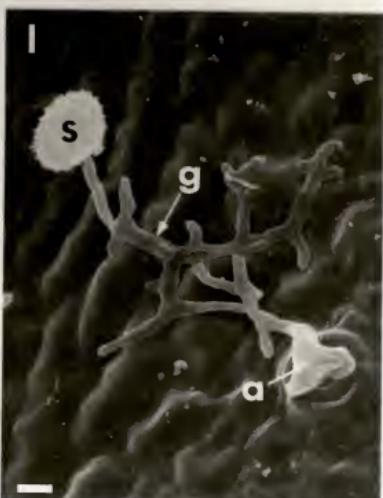
Figure 4 - (J,K). Electron photomicrographs of telia of <u>Cronartium</u>
<u>quercuum</u> f. sp. <u>fusiforme</u> on <u>Quercus</u> <u>rubra</u> leaf tissue:
J) Developing telial column (t) with uredial pustule (u)
adjacent (320 X; bar = 50 µm). K) Mature telial column
with probasidia (p) present. Urediospores (u) are present
on the _eaf surface (700 X; bar = 50 µm).
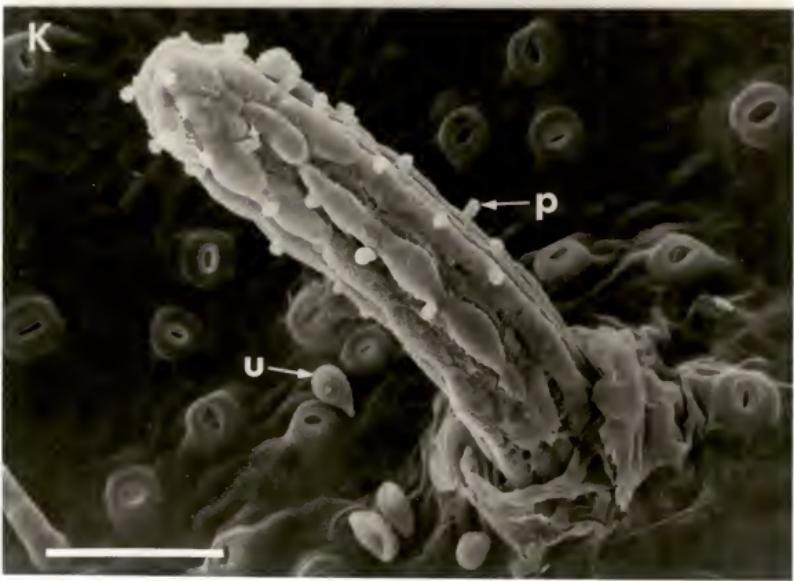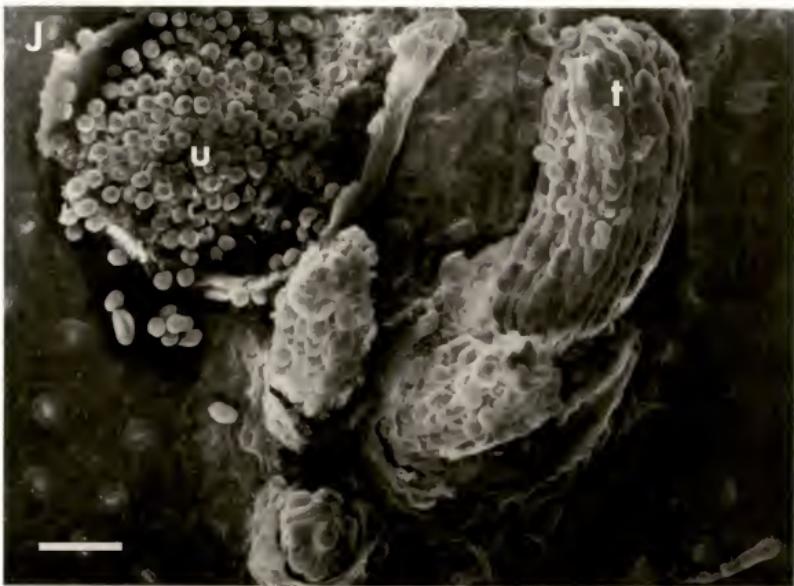
Figure 5 – The effect of temperature on germination following
inoculation with aeciospores of <u>Cronartium</u> <u>quercuum</u> f.
sp. <u>fusiforme</u> on water agar.
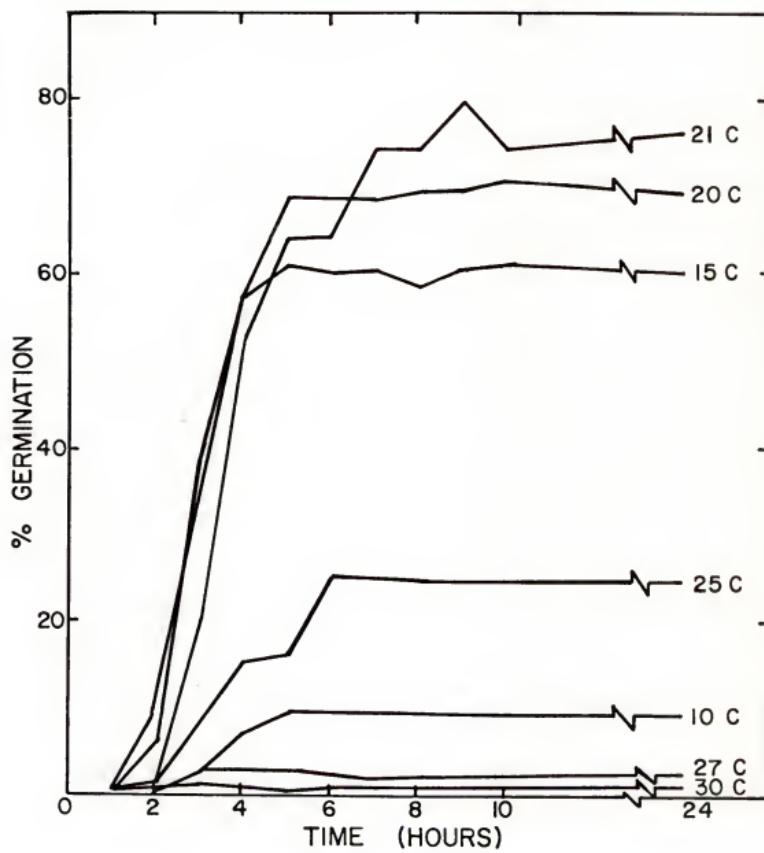
observed while these maxima progressively decreased at temperatures exceeding the optimum.

On both northern red oak leaf discs and the water agar control, maximum aeciospore germination (35%) after 24 hours occurred at 21 C (Fig. 6). Minimal germination of 2 and 3% occurred at 10 an 30 C, respectively. In general, low percentage germination occurred at 10 and 30 C, intermediate germination occurred at 15, 25, and 27 C, and high germination occurred at 20 and 21 C (optimum).

At the optimum temperature (21 C), the theoretical maximum number of aeciospores expected to germinate on water agar was approximately 76% (Table 1). The maximum germination rate at this temperature occurred after 3.5 hours. An additional 1.4 hours (4.9 hours total) were required for the theoretical maximum number of germinated aeciospores to reach 90%. At 15 and 20 C, respectively, the theoretical maximum number of germinating aeciospores progressively increased as compared to temperatures above the optimum when the theoretical maxima decreased. The optimum temperature for the theoretical maximum number of aeciospores expected to germinate (36%) on oak leaf discs was also 21 C (Table 2). The maximum germination rate at this temperature occurred after 5.8 hours while an additional 3.2 hours were required for the theoretical maximum number of germinated aeciospores to reach 90%. As with aeciospores germinated on water agar, the theoretical maximum numbers of aeciospores expected to germinate on oak leaf discs were normally distributed about the optimum.

After 24 hours the percentage of aeciospores which formed appressoria was maximum at 15 C (Fig. 7). After 24 hours at 10, 25, and 30 C,

Figure 6 - The effect of temperature on germination following
inoculation with aeciospores of <u>Cronartium</u> <u>quercuum</u> f.
sp. <u>fusiforme</u> on <u>Quercus</u> <u>rubra</u> leaf discs.

Table 1. The calculated effect of temperature on percentages and rates of germination following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on water agar.

Temp C	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time to 90% Theoretical Maximum $t_{.90}$ (hr)	Proportion of Variation Explained R^2
					Percentage and Rate of Germination[a]		
10	2.26±0.20	9.59±0.10	5.42	2.3	3.30	4.3	.99
15	2.66±0.14	60.11±0.30	39.97	1.2	2.77	3.6	.99
20	1.79±0.08	69.41±0.30	31.06	1.8	2.97	4.2	.99
21	1.57±0.23	74.49±1.40	29.24	1.3	3.50	4.9	.99
25	1.31±0.18	24.47±0.62	6.91	1.8	3.76	5.7	.98
27	*	2.48±0.12	41.79	1.9	1.97	2.0	*
30	*	0.97±0.05	7.40	0.9	0.93	1.0	*

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit:

$$Y = \frac{D}{1 + e^{a-bt}} ;$$

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by 0.10D = $\dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by 0.90D = $\dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation); $\dfrac{Db}{4}$.

* Sufficient information to estimate only D.

Table 2. The calculated effect of temperature on percentages and rates of germination following inoculation with aeciospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Temp C	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time to 90% Theoretical Maximum $t_{.90}$ (hr)	Proportion of Variation Explained R^2
				Percentage and Rate of Germination[a]			
10	*	1.60±0.09	*	1.4	*	2.1	*
15	0.64±0.09	12.93±0.47	2.07	2.0	4.41	8.8	.97
20	0.74±0.07	29.24±0.70	5.41	2.4	5.34	8.2	.99
21	0.67±0.07	34.22±1.53	5.73	2.5	5.81	9.0	.96
25	0.70±0.08	8.41±0.27	0.01	2.7	5.86	8.8	.98
27	1.03±0.23	5.68±0.09	0.01	1.4	3.55	5.6	.94
30	0.61±0.11	2.29±0.09	0.002	0.3	3.39	1.9	.94

a Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit,

$$Y = \frac{D}{1 + e^{a-bt}} \; ;$$

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by $0.10D = \frac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \frac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved to t: $\frac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation): $\frac{Db}{4}$.

* Data had sufficient information to estimate only D with any reasonable degree of accuracy.

Figure 7 – The effect of temperature on appressorial formation
           following inoculation with aeciospores of Cronartium
           quercuum f. sp. fusiforme on Quercus rubra leaf discs.
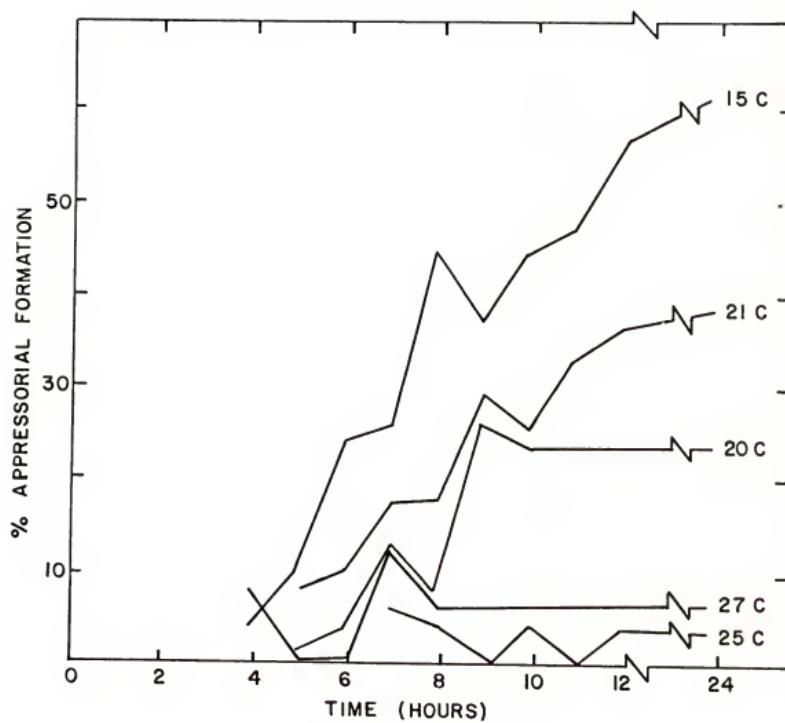
virtually no appressoria had formed. At 15 C the theoretical maximum number of spores to form appressoria was approximately 62% (Table 3). The maximum rate of appressorial formation occurred after 7.4 hours, and an additional 2.1 hours were required to reach 90% of the maximum. At temperatures above the optimum, increasingly fewer numbers of spores were expected to form appressoria and the time to 90% of this theoretical maximum steadily decreased.

Temperature Experiments -- Urediospore Inoculations

The optimum temperature after 24 hours for urediospore germination (71%) on water agar was 18 C (Fig. 8). Similarly high percentages of germination were observed at 15 and 21 C. Approximately 40 to 50% of the urediospores germinated after 24 hours at 10 and 25 C. Minimal germination (5%) occurred at 30 C. The optimum temperature for the theoretical maximum number of urediospores expected to germinate was 18 C (Table 4). The maximum rate of germination occurred after 1.3 hours while an additional 2.1 hours were required for the theoretical maximum number of urediospores expected to germinate to reach 90%. Theoretical maximum numbers of urediospores expected to germinate were distributed about the optimum temperature. The minimum number of spores expected to germinate occurred at 30 C.

The optimum temperature for urediospore germination on oak leaf discs was 18 C (Fig. 9). Similarly high percentages of germination occurred at 15 and 21 C (52 to 60%) but at 10, 25, and 30 C the percent germination was less than 20%. The theoretical maximum numbers of urediospores expected to germinate were normally distributed about the optimum temperature (18 C) while the minimum number of spores expected to germinate occurred at 30 C (Table 5).

Table 3. The calculated effect of temperature on percentages and rates of appressorial formation following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Temp C	Initial Relative Rate b (%/hr)	Percentage and Rate of Formation[a] Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time to 90% Theoretical Maximum $t_{.90}$ (hr)	Proportion of Variation Explained R^2
10		0*					
15	0.63±0.07	62.14±0.03	9.79	8.7	9.05	12.5	.98
20	1.06±0.37	23.98±0.02	6.35	5.3	7.43	9.5	.91
21	0.60±0.09	37.64±0.02	5.65	4.1	7.78	11.5	.98
25		0*					
27	0.80±1.07	6.30±0.02	1.26	1.5	4.26	7.0	.20
30		0*					

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit:

$$Y = \frac{D}{1 + e^{a-bt}} ;$$

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved to t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation): $\dfrac{Db}{4}$.

* Appressorial formation at 10, 25, 30 C was approximately zero.

Figure 8.   The effect of temperature on germination following
            inoculation with urediospores of <u>Cronartium</u> <u>quercuum</u>
            f. sp. <u>fusiforme</u> on water agar.
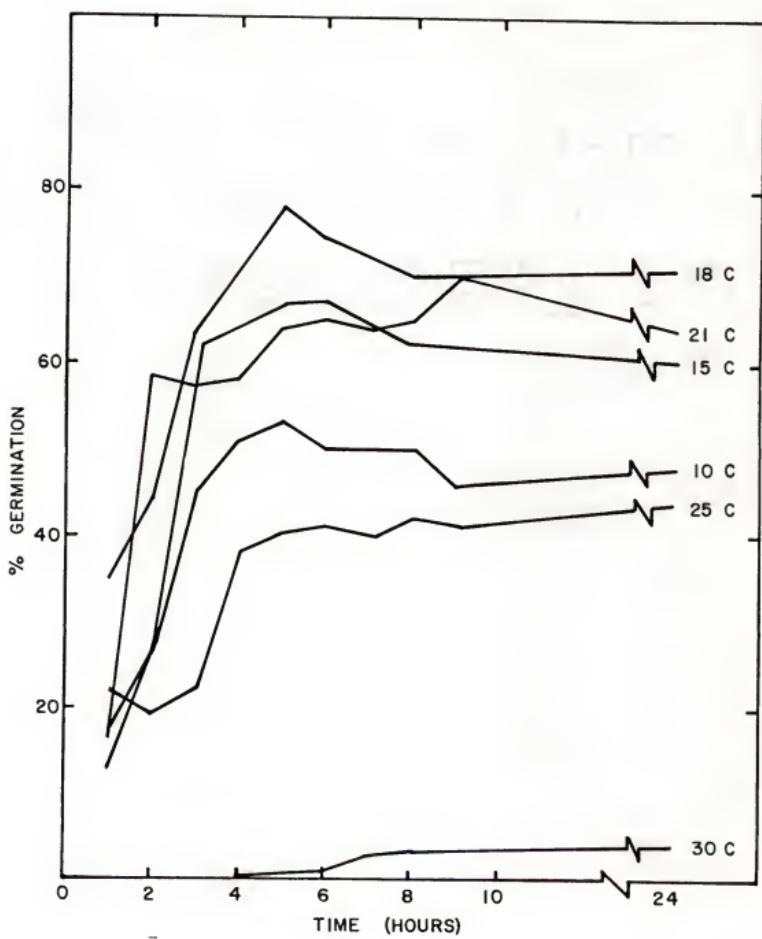
Table 4. The calculated effect of temperature on percentages and rates of germination following inoculation with urediospores of Cronartium quercuum f. sp. fusiforme on water agar.

Temp C	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time to 90% Theoretical Maximum $t_{.90}$ (hr)	Proportion of Variation Explained R^2
			Percentage and Rate of Germination[a]				
10	1.39±0.30	49.69±1.23	17.27	0.08	1.67	3.3	.92
15	3.77±0.09	61.54±1.03	58.00	0.69	1.28	1.8	.96
18	1.01±0.24	73.08±2.00	18.45	0.01	1.31	3.4	.91
21	1.96±0.39	66.50±1.55	32.59	0.96	2.08	3.2	.96
25	0.54±0.18	43.78±2.84	5.91	0.01	1.83	4.2	.82
30	1.40±0.44	3.60±0.23	1.26	4.20	5.79	7.3	.94

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calaculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equat to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivation (of Y with respect to t of the above equation): $\dfrac{Db}{4}$.

Figure 9. The effect of temperature on germination following
          inoculation with urediospores of <u>Cronartium quercuum</u>
   f. sp. <u>fusiforme</u> on <u>Quercus rubra</u> leaf discs.
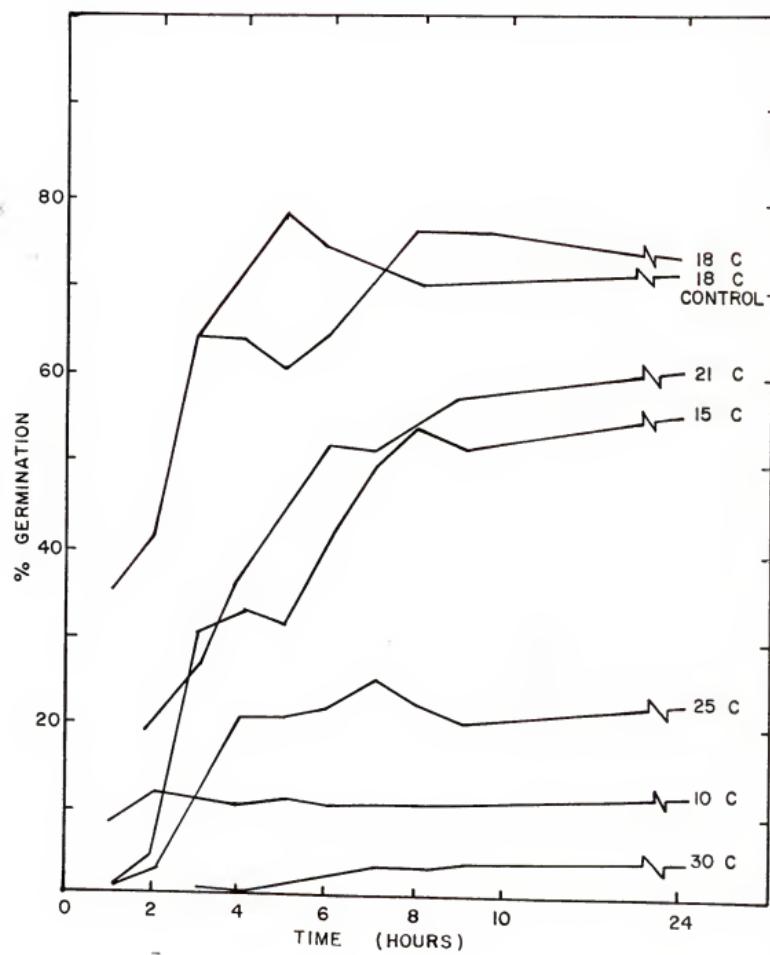
Table 5. The calculated effects of temperature on percentages and rates of germination following inoculation with urediospores of Cronartium quercum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Temp °C	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time of 90% Theoretical Maximum $t_{.90}$ (hr)	Proportion of Variation Explained R^2
			Percentage and Rate of Germination[a]				
10	16.21±0.05	10.85±0.24	43.97	2.9	3.12	3.2	.98
15	0.73±0.07	54.37±1.17	9.92	0.01	2.90	6.0	.99
18	0.51±0.15	74.11±3.57	9.45	0.01	1.14	5.4	.86
21	0.60±0.16	59.11±5.11	8.87	0.06	4.23	7.9	.90
25	0.75±0.26	22.67±1.35	4.25	0.01	2.03	4.9	.82
30	1.41±0.45	3.59±0.22	1.27	4.18	5.79	7.3	.94

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum light,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivation (of Y with respect to t of the above equation) set equal to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect of t of the above equation): $\dfrac{Db}{4}$.

The optimum temperature for appressorial formation form uredio-
spores was also 18 C (Fig. 10). Slightly higher percentages of appres-
sorial formation were observed at 21 and 25 C than at 15 C. No appres-
soria were observed after 24 hours at either 10 or 30 C. The theoreti-
cal maximum appressorial formation (28%) occurred at 18 C and the maxi-
mum formation rate occurred after 5.9 hours (Table 6). An additional
2.1 hours were required before 90% of the theoretical maximum appressor-
ial formation occurred. These theoretical maxima increased until the
optimum temperature was surpassed after which these values decreased.
The maximum rate of appressorial formation decreased to a minimum at
18 C. At temperatures greater than 18 C this rate increased linearly.

Leaf Surface Moisture Experiments -- Aeciospore Inoculations

Percent germination of aeciospores steadily increased with in-
creasing duration of continuous leaf disc moisture, although high germi-
nation levels (> 24%) did not occur until at least 6 hours of moisture
were provided (Fig. 11). A low level of aeciospore germination occurred
(8%) in the absence of continuous surface moisture. This was presumably
due to the inherent moisture levels present around ungerminated spores
or on the leaf surfaces. A minimum of 6 hours at 21 C was required to
attain the highest maximum average percent germination (Table 7). Al-
though the average percent germination increased with increasing dura-
tions of continuous surface moisture, average germination after 5 hours
of moisture was half that at 6 hours. No significant differences in
maximum average germination were observed during 6 to 9 hours of contin-
uous moisture. However, a significant relationship (α = .001) existed

Figure 10 – The effect of temperature on appressorial formation following inoculation with urediospores of Cronartium quercuum f. sp. fusiforme on Quercus rubra leaf discs.

Table 6. The calculated effect of temperature on percentages and rates of appressorial formation following inoculation with urediospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Temp C	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t.10$ (hr)	Time of Maximum Rate (Time to 50% Theoretical Maximum) t_{max} (hr)	Time to 90% Theoretical Maximum $t.90$ (hr)	Proportion of Variation Explained R^2
10		*					
15	3.47±2.03	12.48±0.01	10.83	5.66	6.42	6.9	.94
18	1.03±0.16	28.29±0.01	7.28	3.73	5.86	8.0	.98
21	1.81±0.59	21.99±0.02	9.95	6.98	8.19	9.4	.94
25	1.78±0.36	22.86±0.01	10.17	5.03	6.26	7.5	.98
30		*					

a Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum limit,

b = initial relative rate,

$t.10$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t.90$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation): $\dfrac{Db}{4}$.

* Appressorial formation at 10 and 30 C was approximately zero.

Figure 11 – The effect of continuous leaf surface moisture on germ-
ination following inoculation with aeciospores of
Cronartium *quercuum* f. sp. *fusiforme* on *Quercus* *rubra*
leaf discs.

Table 7. The effect of duration of continuous leaf surface moisture on percentages and rates of germination following inoculation with aeciospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quecus rubra* seedlings.

Leaf Moisture Duration LMD (hr)	Maximum[a] Average Observed M (%)	Percentage and Rate of Germination		Predicted[b] Germination As a Function of Leaf Moisture (%)
		Time to 10% Maximum Average Observed (hr)	Time to 90% Maximum Average Observed (hr)	
0	9.00	< 1.0	3.0	4.43
1	12.00	< 1.0	3.0	9.28
2	12.00	< 1.0	3.0	14.14
3	18.00	< 1.0	3.0	19.00
4	23.00	< 1.0	5.0	23.85
5	24.00	< 1.0	5.0	28.71
6	48.00	< 1.0	6.0	33.57
7	44.00	< 1.0	6.0	38.42
8	46.00	< 1.0	6.0	43.28
9	46.00	< 1.0	6.0	48.14

a   The maximum average observed (M) was the highest average percentage germination average at each moisture period.

b   Regression of percent germination of leaf moisture duration (LMD) indicated a significant (α = .001) linear response of the form: % Germination = 4.43 + 4.86 (LMD). Quadratic response was not significant from hours 8 to 24. (r^2 = .88).

between the percentage of aeciospore germination and the regression of leaf surface moisture duration. The response progressed linearly as moisture duration increased.

The greatest aeciospore appressorial formation (38%) occurred after 24 hours of leaf surface moisture (Fig. 12). No appressoria were formed when continuous leaf surface moisture was 0 to 2 hours in duration. The percent appressorial formation steadily increased after 2 hours of continuous leaf surface moisture. The maximum average appressorial formation (38%) occurred after a minimum of 9 hours of continuous leaf surface moisture (Table 8). A significant relationship (α = .001) existed between the percentage of aeciospore appressorial formation and the regression of leaf surface moisture duration. The response progressed quadratically as moisture duration increased. Despite little difference in maximum average appressorial formation at 9 and 24 hours, the actual maximum appressorial formation occurred after the longest exposure to continuous moisture, i.e., 24 hours (Fig. 12).

In the first experiment with interrupted leaf surface moisture, 26% of the aeciospores germinated and 30% of these formed appressoria at 2 hours of wetness plus 6 hours of drying plus 16 hours of wetness (Fig. 13). In the second experiment, 22% of the spores germinated and 3.7% of these formed appressoria at 6 hours of wetness plus 2 hours of dryness plus 16 hours of wetness. Only 25% of the spores germinated on water agar which was used as a spore viability check. Little difference was observed between the two experiments in either germination (Table 9) or appressorial formation (Table 10).

Figure 12 – The effect of continuous leaf surface moisture on
appressorial formation following inoculation with
aeciospores of <u>Cronartium</u> <u>quercuum</u> f. sp. <u>fusiforme</u>
on <u>Quercus</u> <u>rubra</u> leaf discs.
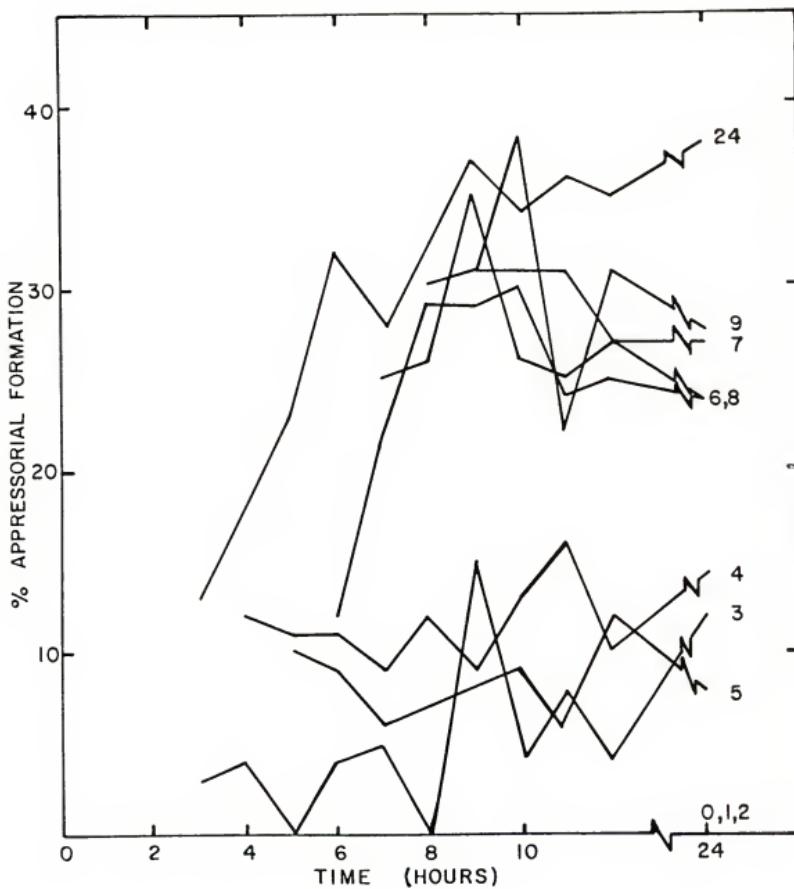
Table 8. The effect of duration of continuous leaf surface moisture on percentages and rate of appressorial formation following inoculation with urediospores of *Cronartium quercum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Leaf Moisture Duration LMD (hr)	Percentage and Rate of Appressorial Formation			
	Maximum[a] Average Observed M (%)	Time to 10% Maximum Average Observed (hr)	Time to 90% Maximum Average Observed (hr)	Predicted[b] Appressorial Formation as a Function of Leaf Moisture (%)
0	0	–	–	– 5.50*
1	0	–	–	– 0.35*
2	0.1	–	–	4.50
3	15.0	< 3.0	5.0	9.06
4	16.0	< 3.0	5.0	13.31
5	12.0	< 3.0	5.0	17.27
6	30.0	< 3.0	8.0	20.93
7	35.0	< 3.0	8.0	24.29
8	31.0	< 3.0	8.0	27.35
9	38.0	< 3.0	8.0	30.11
24	38.0	< 3.0	8.0	35.68

a   The maximum average observed (M) was the highest average percentage appressorial formation at each moisture period.

b   Regression of percent appressorial formation of leaf moisture duration (LMD) indicated a significant ($\alpha = .001$) quadratic regression of the form: % Appressorial formation = -5.49 + 5.30 (LMD) - 0.15(LMD^2), based on data 8 to 24 hours. ($r^2 = .86$).

*   Negative predicted values should be interpreted as zero percentage appressorial formation.

Figure 13 - The effect of an interruption in the period of leaf
surface moisture on germination and appressorial
formation following inoculation with aeciospores of
Cronartium quercuum f. sp. fusiforme on Quercus
rubra leaf discs.

68
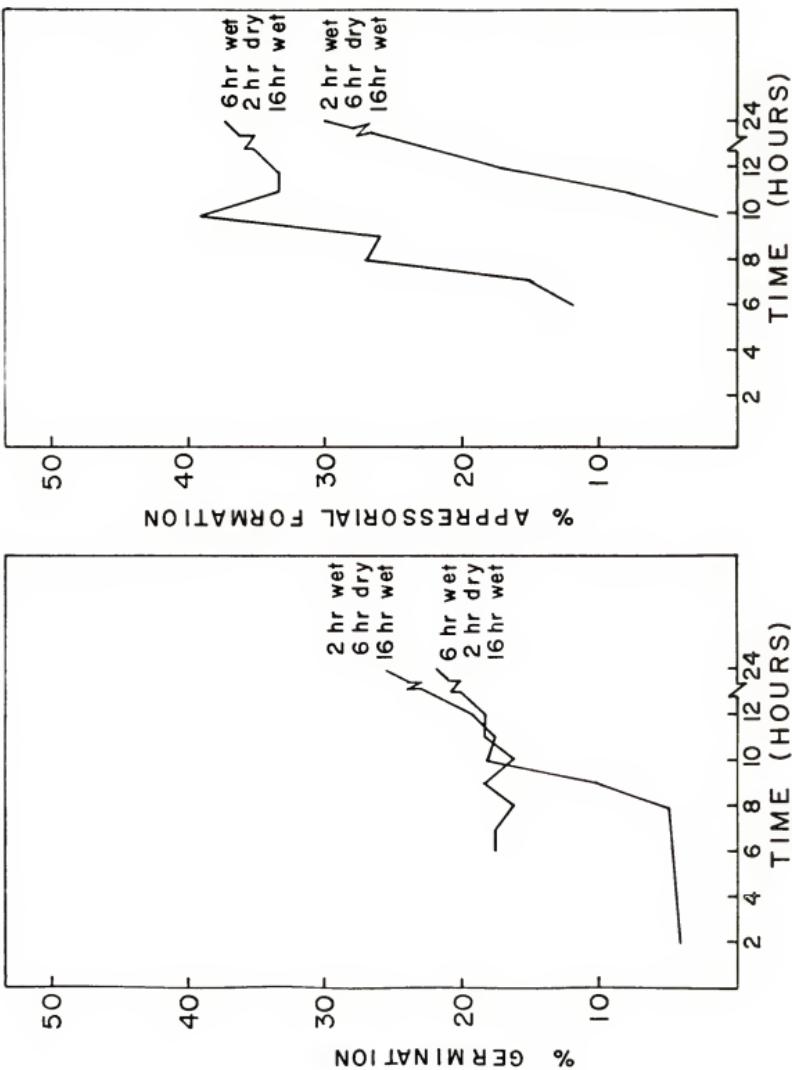
Table 9. Influence of an interruption of leaf surface moisture on percentage germination following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Moisture Period (hr)	Mean Germination[a] (%)
2 wet – 6 dry – 16 wet	26.00
6 wet – 2 dry – 16 wet	22.00

a Average germination after 24 hours.

Table 10. Influence of an interruption of leaf surface moisture on percentage appressorial formation following inoculation with aeciospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Moisture Period (hr)	Mean Appressorial Formation[a] (%)
2 wet – 6 dry – 16 wet	30.6
6 wet – 2 dry – 16 wet	38.2

[a] Average appressorial formation after 24 hours.

Leaf Surface Moisture Experiments -- Urediospore Inoculations

Minimal urediospore germination (3%) occurred in the absence of leaf surface moisture (Fig. 14). Percent germination steadily increased with increasing duration of surface moisture with maximum germination (70%) occurring after 24 hours of surface moisture. The maximum average germination (68%) was reached after only 8 hours of continuous leaf surface moisture (Table 11). Less than 1% of the urediospores formed appressoria at less than 1 hour of surface moisture (Fig. 15). Appressorial formation progressively increased from 1% at 2 hours moisture to 28% at 24 hours moisture. The maximum average percent appressorial formation also occurred after 24 hours of surface moisture (Table 12). A significant relationship (α = .001) existed between the percentage of urediospore germination and appressorial formation and the regression of leaf surface moisture duration. The response progressed quadratically as moisture duration increased.

In the first experiment with interrupted leaf surface moisture, 55% of the urediospores germinated and 4% of these formed appressoria at 1 hour of wetness plus 1 hour of drying plus 16 hours of wetness (Fig. 16). In the second experiment, 57% of the spores germinated and 2% of these formed appressoria at 1 hour of wetness plus 3 hours drying plus 20 hours wetness (Fig. 16). However, 71% of the urediospores germinated on the water agar check plate. Little difference in either germination (Table 13) or appressorial formation (Table 14) was observed between the two experiments.

Figure 14 – The effect of continuous leaf surface moisture on germination following inoculation with urediospores of _Cronartium quercuum_ f. sp. _fusiforme_ on _Quercus rubra_ leaf discs.

Table 11. The effect of duration of continuous leaf surface moisture on percentages and rates of germination following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Leaf Moisture Duration LMD (hr)	Maximum[a] Average Observed M (%)	Percentage and Rate of Germination		Predicted[b] Germination As a Function of Leaf Moisture (%)
		Time to 10% Maximum Average Observed (hr)	Time to 90% Maximum Average Observed (hr)	
0	4.0	< 1.0	1.0	6.71
1	18.0	< 1.0	1.0	21.24
2	47.0	< 1.0	2.0	33.59
3	51.0	< 1.0	2.0	43.76
4	57.0	< 1.0	2.0	51.73
5	52.0	< 1.0	2.0	57.52
6	55.0	< 1.0	2.0	61.12
7	66.0	< 1.0	3.0	62.54
8	68.0	< 1.0	3.0	61.77

a   The maximum average observed (M) was the highest average percentage germination at each moisture period.

b   Regression of percent germination of leaf moisture duration indicated a significant (α = .001) quadratic regression of the form: % Germination = 6.70 + 15.62 (LMD) - 1.09(LMD^2) based on data from 8 to 24 hours. (r^2 = .90).

Figure 15 – The effect of continuous leaf surface moisture on
appressorial formation following inoculation with
urediospores of Cronartium quercuum f. sp. fusiforme
on Quercus rubra leaf discs.
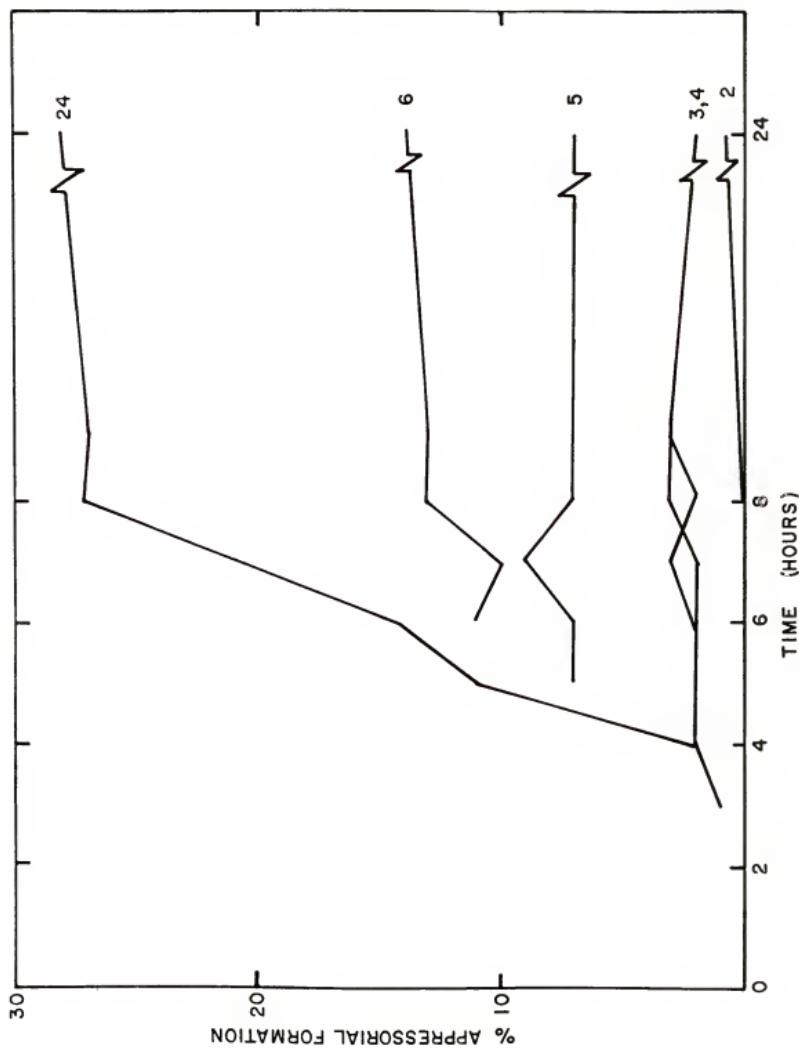
Table 12. The effect of duration of continous leaf surface moisture on percentages and rates of appressorial formation following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Leaf Moisture Duration LMD (hr)	Maximum[a] Average Observed M (%)	Percentage and Rate of Appressorial Formation		Predicated[b] Appressorial Formation as a Function of Leaf Moisture (%)
		Time to 10% Maximum Average Observed (hr)	Time to 90% Maximum Average Observed (hr)	
0	0	< 3.0	–	– 1.83*
1	0	< 3.0	–	0.06
2	1.0	< 3.0	3.0	1.94
3	3.0	< 3.0	3.0	3.74
4	3.0	< 3.0	3.0	5.47
5	9.0	< 3.0	5.0	7.15
6	13.0	< 3.0	5.0	8.76
24	28.0	< 3.0	8.0	27.48

a   The maximum average observed (M) was the highest average percentage appressorial formation at each moisture period.

b   Regression of percent appressorial formation of leaf moisture duration indicated a significant (α = .001) quadratic regression of the form: % Appressorial formation = -1.83 + 1.95(LMD) - 0.03(LMD^2) based on data from 8 to 24 hours. (r^2 = .94).

*   Negative predicted values should be interpreted as zero percent appressorial formation.

Figure 16 – The effect of an interruption in the period of leaf
surface moisture on germination and appressorial
formation following inoculation with urediospores of
Cronartium quercuum f. sp. fusiforme on Quercus rubra
leaf discs.
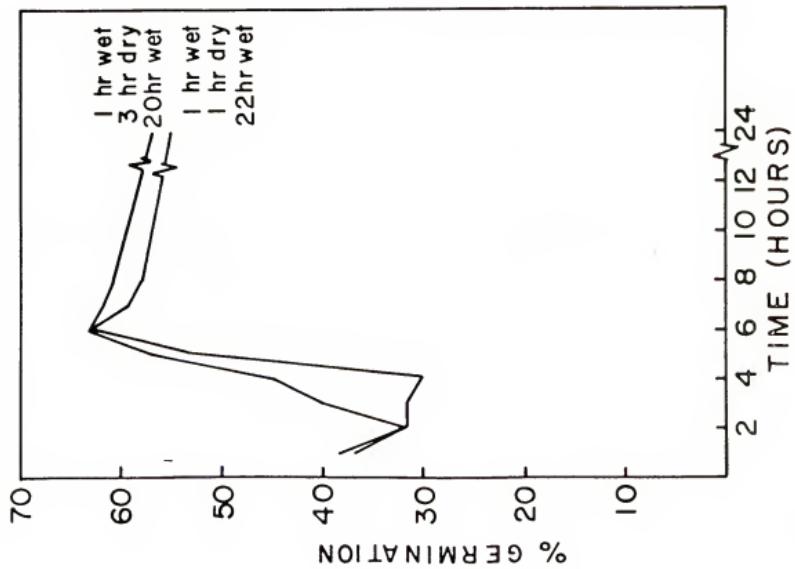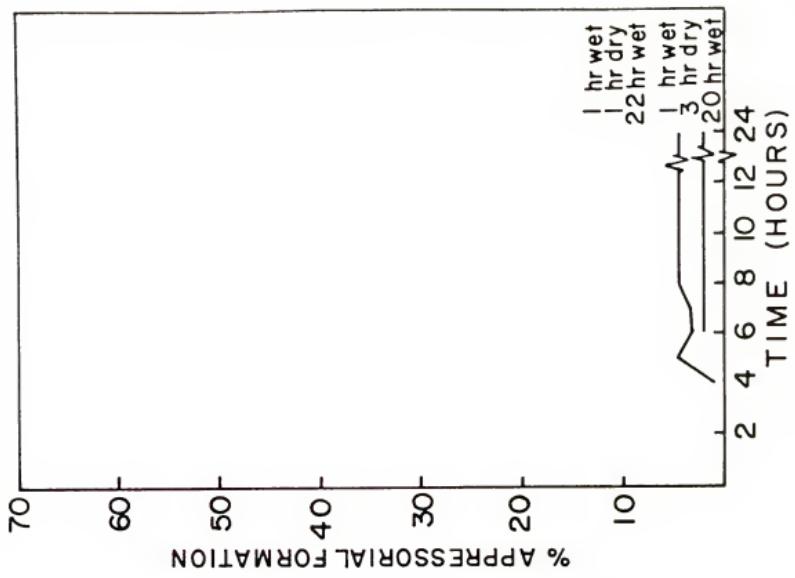
Table 13. Influence of an interruption of leaf surface moisture on percentage germination following inoculation with urediospores of Cronartium _quercuum_ f. sp. _fusiforme_ on leaf discs of _Quercus rubra_ seedlings.

Moisture Period (hr)	Mean Germination[a] (%)
1 wet – 1 dry – 22 wet	54.7
1 wet – 3 dry – 20 wet	56.7

[a] Average germination after 24 hours.

Table 14. Influence of an interruption of leaf surface moisture on percentage appressorial formation following inoculation with urediospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Moisture Period (hr)	Mean Appressorial Formation [a] (%)
1 wet – 1 dry – 22 wet	4.3
1 wet – 3 dry – 20 wet	2.3

a   Average appressorial formation after 24 hours.

Leaf Age Experiments -- Aeciospore Inoculations

Aeciospore germination was approximately 20% at all leaf ages of 2, 4, 5, 6, 11, and 16 days (Fig. 17). Percent appressorial formation was slightly more variable and increased with leaf age; for example, 36% of the aeciospores formed appressoria at 2 days compared to 42% at 16 days. The theoretical maximum aeciospore germination (25%) occurred at 5 days (Table 15). The theoretical maximum appressorial formation (37%) occurred when leaves were 11 days old (Table 16).

Leaf Age Experiments -- Urediospore Inoculations

Percent urediospore germination was approximately 53% at all leaf ages of 2, 4, 9, 13, and 20 days (Fig. 18). No significant difference in theoretical maximum germination was observed among all leaf ages (Table 17). Percent appressorial formation was also consistent (12 to 14%) across the leaf age classes. Maximum percent germination (54%) occurred after 20 days and maximum percent appressorial formation (14%) occurred at 4 and 13 days (Fig. 18). The theoretical maximum percent germination (51%) also occurred at 20 days (Table 17). The theoretical maximum appressorial formation occurred when leaves were 4 days old (Table 18).

Quantification of Inoculum

Uredial sori developed 6 days after aeciospore inoculation of 2-, 5-, and 6-day-old leaves (Fig. 19). No sori developed on the 8- or 11-day-old leaves. The latent period for uredial sori was approximately 7 days. The maximum average number of uredial sori per cm^2 (0.5) of leaf surface occurred on 5-day-old leaves with only 0.2 and 0.1 sori appearing on 2- and 6-day-old leaves respectively (Tables 19 and 20).

Figure 17 – The effect of leaf age on germination and appressorial
formation following inoculation with aeciospores of
Cronartium quercuum f. sp. _fusiforme_ on _Quercus rubra_
leaf discs.
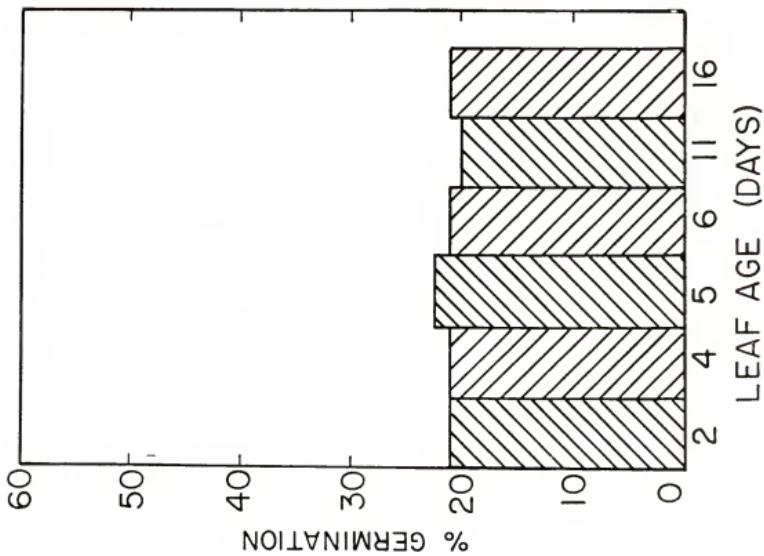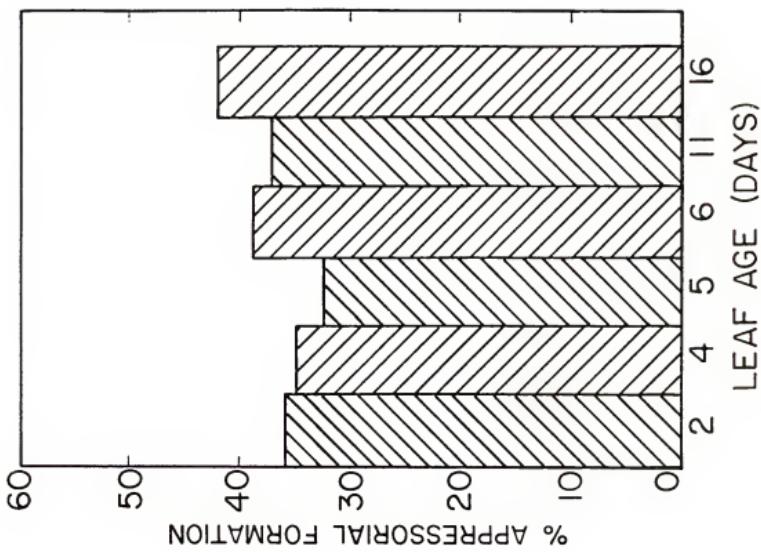
Table 15. The calculated effect of leaf age on percentages and rates of germination following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Leaf Age (day)	Percentage and Rate of Germination[a]						
	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum t_{10} (hr)	Time to Maximum Rate t_{max} (hr)	Time to 90% Theoretical Maximum t_{90} (hr)	Proportion of Variation Explained R^2
2	0.72±0.09	20.46±0.67	3.68	2.7	5.82	8.8	.98
4	0.59±0.09	21.70±0.09	3.20	2.4	6.21	9.7	.96
5	0.77±0.13	23.50±0.94	4.52	2.5	5.24	8.0	.96
6	0.79±0.08	21.73±0.48	4.29	2.3	5.01	7.8	.99
11	0.69±0.07	19.85±0.53	3.42	2.0	5.22	8.4	.98
16	0.67±0.09	21.54±0.74	3.61	1.7	5.00	8.2	.97

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation: $\dfrac{Db}{4}$.

Table 16. The calculated effect of leaf age on percentages and rates of appressorial formation following inoculation with aeciospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Leaf Age (day)	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Percentage and Rate of Appressorial Formation [a]				Proportion of Variation Explained R^2
			Rate at Maximum Point r_{max} (%/hr)	Time to Theoretical Maximum $t.10$ (hr)	Time of Maximum Rate t_{max} (hr)	Time to 90% Theoretical Maximum $t.90$ (hr)	
2	0.92±0.12	33.36±0.01	7.67	4.6	6.75	9.1	.86
4	2.38±0.46	32.31±0.01	19.22	5.6	6.71	7.4	.98
5	1.76±0.39	30.85±0.01	19.22	5.5	6.74	7.4	.98
6	1.11±0.27	32.74±0.02	9.09	4.6	6.64	8.6	.96
11	0.46±0.26	36.47±0.09	4.19	2.8	7.61	12.3	.56
16	2.13±0.86	35.05±0.02	18.66	5.4	6.50	7.5	.92

[a] Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + 3^{a-bt}}$;

D = theoretical maximum limit,

b = initial relative rate,

$t.10$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t.90$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved to t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation): $\dfrac{Db}{4}$.

Figure 18 – The effect of leaf age on germination and appressorial formation following inoculation with urediospores of *Cronartium quercuum* f. sp. *fusiforme* on *Quercus rubra* leaf discs.
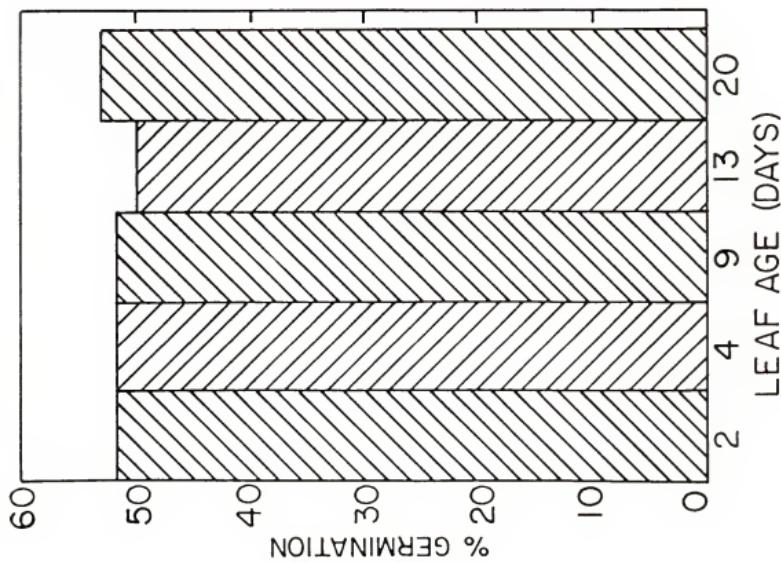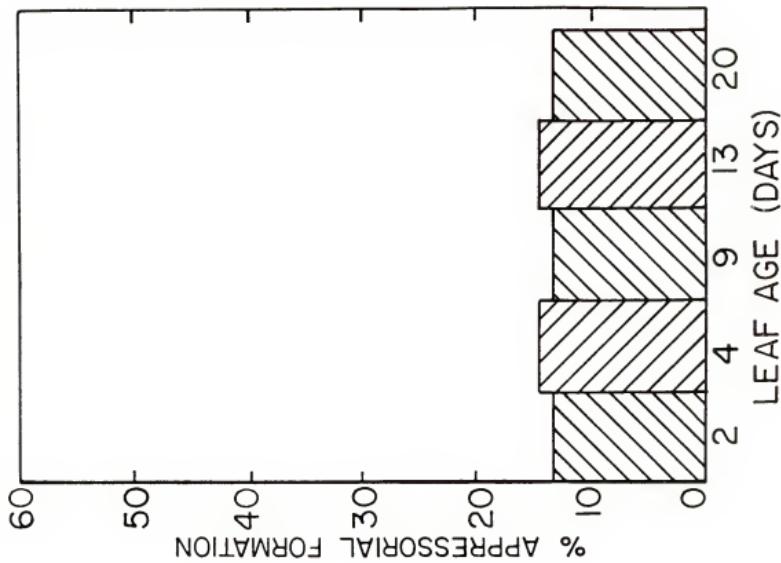
Table 17. The calculated effect of leaf age on percentage of germination following inoculation with urediospores of Cronartium quercuum f. sp. fusiforme on leaf discs of Quercus rubra seedlings.

Percentage of Germination	
Leaf Age (day)	Theoretical Maximum D (%)
2	47.3 ± 1.09
4	49.9 ± 0.63
9	49.9 ± 0.62
13	49.2 ± 1.19
20	49.7 ± 1.36

a Non-linear regression analysis was conducted with data to estimate the parameter (i.e., D) which yields the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum limit.

Data was insufficient to estimate model parameters a and b.

Table 18. The calculated effect of leaf age on percentages and rates of appressorial formation following inoculation with urediospores of *Cronartium quercuum* f. sp. *fusiforme* on leaf discs of *Quercus rubra* seedlings.

Leaf Age	Initial Relative Rate b (%/hr)	Theoretical Maximum D (%)	Percentage and Rate of Appressorial Formation[a]				Proportion of Variation Explained R^2
			Rate at Maximum Point r_{max} (%/hr)	Time to 10% Theoretical Maximum $t_{.10}$ (hr)	Time of Maximum Rate t_{max} (hr)	Time to 90% Theoretical Maximum $t_{.90}$ (hr)	
2	1.16±0.23	13.49±0.01	3.91	3.4	5.37	7.2	.97
4	1.05±0.21	14.85±0.01	3.90	2.6	4.67	6.8	.97
9	1.66±0.16	12.76±0.002	5.30	3.4	4.73	6.0	.99
13	0.95±0.14	13.60±0.01	3.23	2.8	5.18	7.5	.98
20	1.61±0.39	12.83±0.01	5.16	4.7	6.07	7.5	.97

a   Non-linear regression analysis was conducted on data to estimate the parameters (i.e., D and b) which yield the curve of best fit: $Y = \dfrac{D}{1 + e^{a-bt}}$;

D = theoretical maximum limit,

b = initial relative rate,

$t_{.10}$ = time to 10% theoretical maximum, calculated by $0.10D = \dfrac{D}{1 + e^{a-bt}}$,

$t_{.90}$ = time to 90% theoretical maximum, calculated by $0.90D = \dfrac{D}{1 + e^{a-bt}}$,

t_{max} = the second derivative (of Y with respect to t of the above equation) set equal to zero and solved for t: $\dfrac{a}{b}$,

r_{max} = maximum of the first derivative (of Y with respect to t of the above equation): $\dfrac{Db}{4}$.

Figure 19 - The effect of leaf age on the number of sori per cm^2 produced following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on 2- to 11-day-old Quercus rubra seedlings.
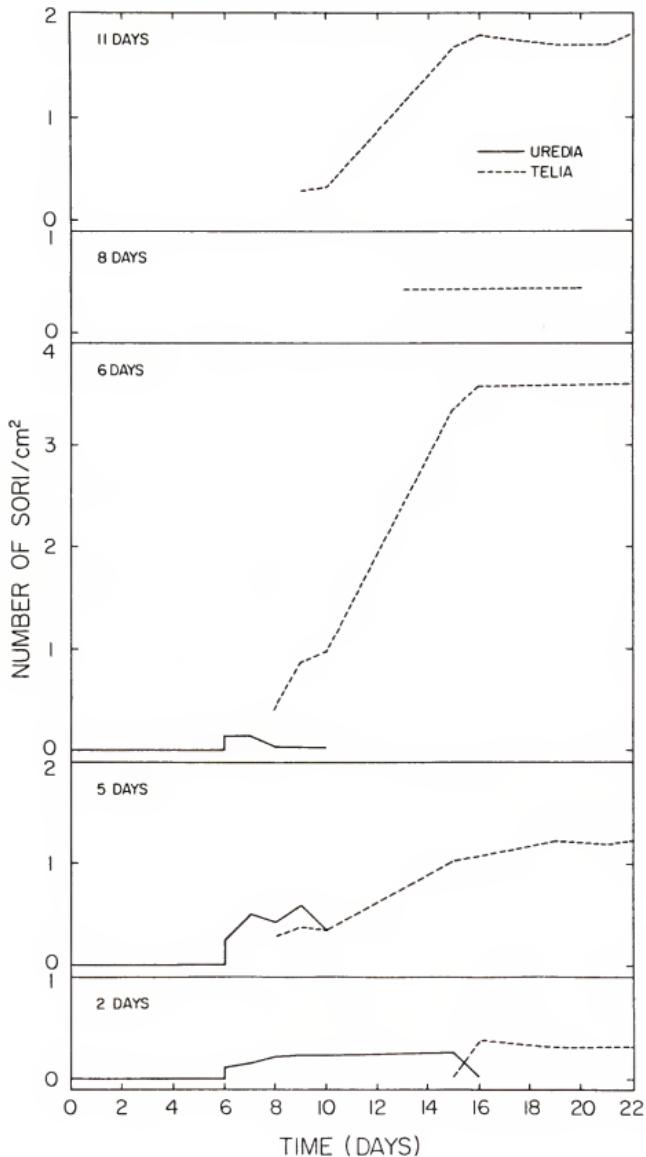
Table 19. The effect of leaf age on the cumulative number of sori per cm² produced following inoculation with aeciospores of Cronartium quercuum f. sp. fusiforme on 2- to 16-day-old leaves of Quercus rubra seedlings.

Leaf Age (day)	Day After Inoculation									
	6	7	8	9	10	15	16	19	21	22
	Sori per cm² [a]									
2	.11u	.14u	.21u	.23u	.21u	.25u .02t	.01u .36t	.30t	.30t	.30t
5	.26u	.51u	.42u .28t	.58u .37t	.33u .33t	1.02t	1.02t	1.23t	1.21t	1.23t
6	.14u	.15u	.04u .42t	.03u .86t	.03u .98t	3.35t	3.56t	3.56t	3.56t	3.56t
8	0	0	0	0	0	.44t	.44t	.44t	.44t	.44t
11	0	0	0	.23t	.32t	1.67t	1.77t	1.70t	1.70t	1.77t
16	0	0	0	0	0	0	0	0	0	0

a   u = uredial sori, t = telial sori.

Table 20. The effect of leaf age on number of uredia and telia pro-
duced following inoculation with aeciospores of _Cronartium_
quercuum f. sp. _fusiforme_ on 2- to 11-day-old leaves of
Quercus _rubra_ seedlings.

	Age of Seedlings (days)	Average Number of Sori $(Sori/cm^2)$
Uredia	2	0.17 ± 0.03 A[a]
	5	0.42 ± 0.06 B
	6	0.08 ± 0.03 A
Telia	2	0.26 ± 0.06 A
	5	0.84 ± 0.15 A, B
	6	2.48 ± 0.51 C
	8	0.44 ± 0 A
	11	1.32 ± 0.26 B

a Means accompanied by different letters are statistically different
at the α = .05 level as determined by the Student T-Test.

Telial columns appeared 8 to 9 days after inoculation of 5-, 6-, and 11-day-old leaves (Fig. 19). Telial columns appeared only after 15 days when 2- and 8-day-old oak leaves were inoculated. The maximum number of telial columns per cm^2 occurred on leaves that were 6 days old when inoculated (Table 20).

An aeciospore inoculum efficiency schedule based upon uredial and telial formation was calculated from inoculation of 2-, 5-, 6-, 8-, and 11-day-old oak leaves (Table 21). An average of 1.07 telial sori per cm^2 of leaf surface developed 20 days after inoculation (1000 ± 100 aeciospores/cm^2).

A temporal sequence for infection and disease development following aeciospore inoculations of northern red oak seedlings is described in Fig. 20. The first incubation period is defined as the period from inoculation to the production of immature uredia which occurred in 6 days. The first latent period (inoculation through mature uredia) required an additional 24 hours. The second incubation period (inoculation through immature telia) required 8 days while the second latent period (inoculation through mature telia) also required an additional day.

Uredial pustules developed in 5 days after urediospore inoculation (1000 ± 100 spores/cm^2) of 2-, 5-, 7-, and 9-day-old leaves (Fig. 21). No uredial pustules developed on 11-day-old leaves. The latent period for uredial pustules was approximately 6 days. The average number of uredial sori per cm^2 was greatest on 2-day-old leaves and was progressively less on 5-, 7-, and 9-day-old leaves, respectively (Tables 22 and 23).

Table 21. Inoculum efficiency following inoculation with aeciospores of _Cronartium quercuum_ f. sp. _fusiforme_ on 2- to 11-day-old of _Quercus rubra_ seedlings maintained in a growth chamber at 21° C.

Time (day)	Process	Infection Units (per cm^2)
0	Inoculation	1000 spores $\pm$ 100
1	Germination	340 spores $\pm$ 20
.1	Appressoria formation	210 spores $\pm$ 10
6 – 16	Uredial Production	0.22 sorus $\pm$ .06
8 – 20	Telial Production	1.07 sori $\pm$ .25

Figure 20 - Time of occurrence for infection and disease develop-
ment following inoculation with aeciospores of
Cronartium quercuum f. sp. fusiforme on Quercus rubra
seedlings maintained at optimal temperature and
moisture conditions for the pathogen.

AECIOSPORES

INOCULATION	GERMINATION	APPRESSORIAL FORMATION	IMMATURE UREDIA	MATURE UREDIA	IMMATURE TELIA	MATURE TELIA
0	5 hr	6 hr	6 day	7 day	8 day	9 day

INCUBATION PERIOD

LATENT PERIOD

INCUBATION PERIOD

LATENT PERIOD
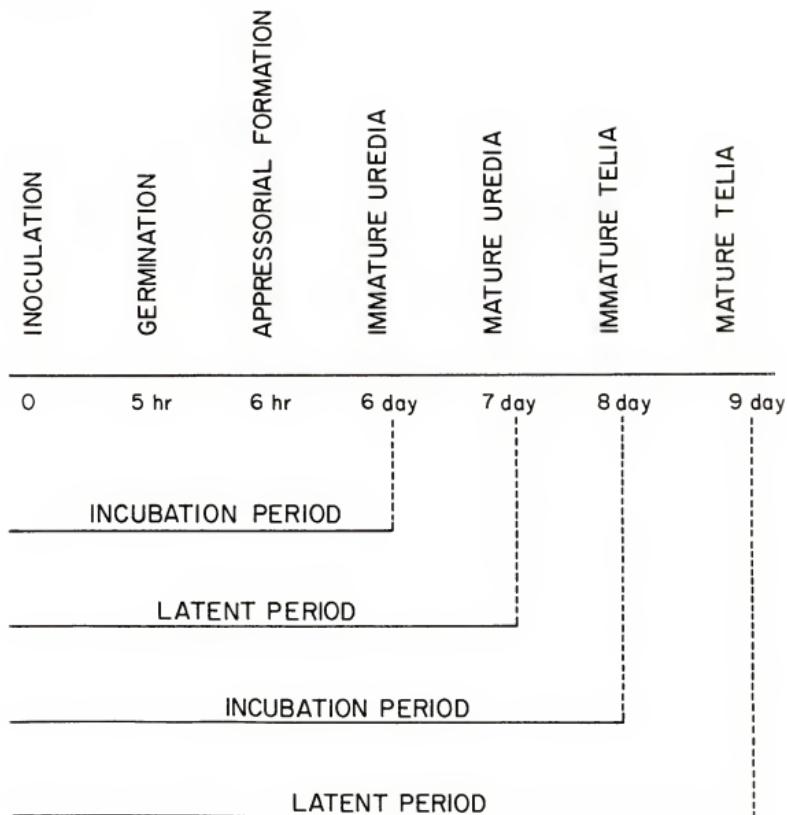
Figure 21 – The effect of leaf age on the number of sori per cm^2 produced following inoculation with urediospores of <u>Cronartium</u> <u>quercuum</u> f. sp. <u>fusiforme</u> on 2- to 11-day-old <u>Quercus</u> <u>rubra</u> seedlings.
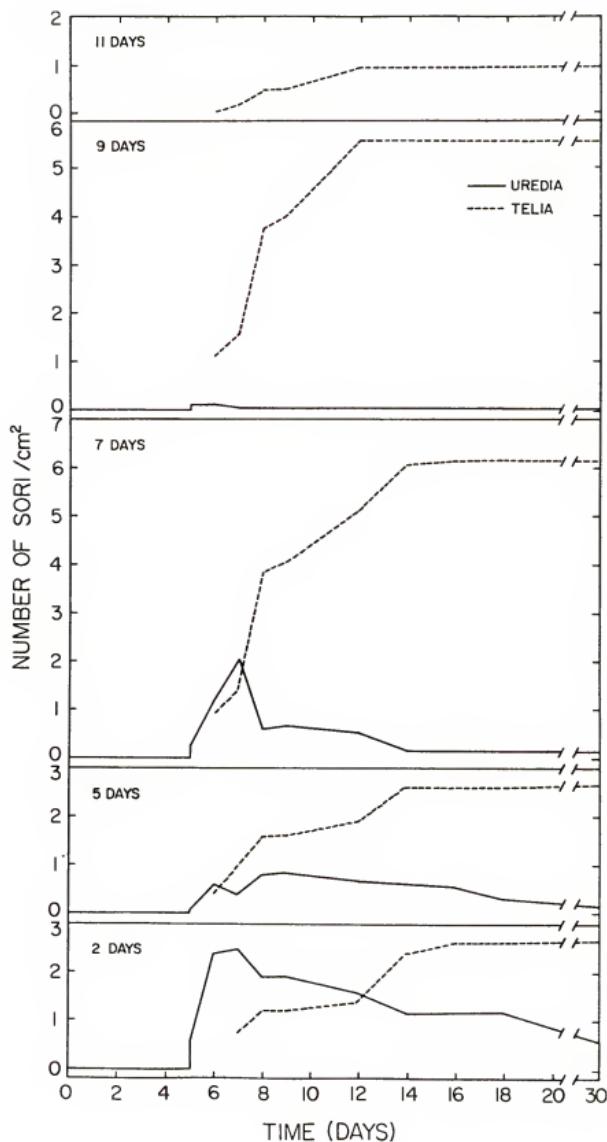
Table 22. The effect of leaf age on the number of sori per cm^2 produced following inoculation with uredio-spores of Cronartium quercuum f. sp. fusiforme on 2- to 14-day-old leaves of Quercus rubra seedlings.

Leaf Age (day)	Day After Inoculation									
	5	6	7	8	9	12 [a]	14	16	18	30
	\-\-\-\-\-\-\-\-\-\-\-\-\- Sori per cm^2 \-\-\-\-\-\-\-\-\-\-\-\-\-									
2	0.61u	2.41u	2.49u 0.65t	1.95u 1.23t	1.05u 1.23t	1.53u 1.42t	1.23u 2.41t	1.23u 2.57t	1.23u 2.57t	0.57u 2.57t
5	0.08u	0.59u 0.41t	0.36u 1.04t	0.77u 1.56t	0.83u 1.65t	0.72u 1.92t	0.66u 2.64t	0.54u 2.64t	0.34u 2.64t	0.14u 2.69t
7	0.27u	1.25u 0.93t	2.07u 1.41t	0.61u 3.77t	0.66u 4.06t	0.66u 5.12t	0.50u 6.05t	0.19u 6.18t	0.19u 6.18t	0.08u 6.18t
9	0.07u	0.07u 1.18t	0.03u 1.64t	0.02u 3.76t	0.03u 4.05t	0.03u 5.62t	0.03u 5.62t	0.03u 5.62t	0.02u 5.62t	0.02u 5.62t
11	0u 0t	0.01t	0.21t	0.47t	0.50t	0.97t	0.97t	0.97t	0.97t	0.97t
14	0	0	0	0	0	0	0	0	0	0

a   u = uredial sori; t = telial sori.

Table 23. The effect of leaf age on number of uredia and telia pro-
          duced following inoculation with urediospores of <u>Cronartium</u>
          <u>quercuum</u> f. sp. <u>fusiforme</u> on 2- to 11-day-old leaves of
   <u>Quercus</u> <u>rubra</u> seedlings.

	Age of Seedling (days)	Average Number of Sori (Sori/cm^2)
Uredia	2	1.52 $\pm$ 0.21 A[a]
	5	0.50 $\pm$ 0.08 B
	7	0.60 $\pm$ 0.20 B
	9	0.04 $\pm$ 0.01 C
Telia	2	1.83 $\pm$ 0.28 A
	5	1.91 $\pm$ 0.27 A
	7	4.43 $\pm$ 0.69 B
	9	4.30 $\pm$ 0.60 B
	11	0.67 $\pm$ 0.13 A

a Means accompanied by different letters are statistically different
   at the α = .05 level as determined by the Student T-Test.

Telial columns appeared 6 days after urediospore inoculation of 5-, 7-, 9-, and 11-day-old leaves (Fig. 21 and Table 22). Telia appeared on 2-day-old leaves after 7 days. The average number of telial sori per cm^2 increased to a maximum (4.4) on 7-day-old leaves and was progressively less on 9- and 11-day-old leaves.

A urediospore inoculum efficiency schedule was calculated from inoculations of 2-, 5-, 7-, 9-, and 11-day-old oak leaves (Table 24). An average of 2.63 telial sori/cm^2 of leaf surface developed 30 days after inoculation (1000 $\pm$ 100 spores/cm^2).

A temporal sequence of urediospore infection and disease development in inoculated oak leaves is presented in Fig. 22. The first incubation period (inoculation through production of immature uredia) required 5 days with an additional day required for production of mature uredia (first latent period). The second incubation period (inoculation through production of immature telia) lasted 6 days with an additional day required for production of mature telia (latent period).

Table 24. Inoculum efficiency following inoculation with urediospores of _Cronartium quercuum_ f. sp. _fusiforme_ on 2- to 11-day-old leaves of _Quercus rubra_ seedlings maintained in a growth chamber at 21° C.

Time (day)	Process	Infection Units (per cm^2)
0	Inoculation	1000 spores $\pm$ 100
1	Germination	740 spores $\pm$ 40
1	Appressorial formation	207 spores $\pm$ 10
5 – 30	Uredial production	0.66 sorus $\pm$ .15
6 – 30	Telial production	2.63 sori $\pm$ .35

Figure 22 – Times of occurrence for infection and disease develop-
ment following inoculation with urediospores of
Cronartium quercuum f. sp. *fusiforme* on *Quercus rubra*
seedlings maintained at optimal temperature and
moisture conditions for the pathogen.

UREDIOSPORES

| INOCULATION	GERMINATION	APPRESSORIAL FORMATION	IMMATURE UREDIA	MATURE UREDIA	IMMATURE TELIA	MATURE TELIA
0	2 hr	4 hr	5 day	6 day	6 day	7-8 day

INCUBATION PERIOD

LATENT PERIOD

INCUBATION PERIOD

LATENT PERIOD
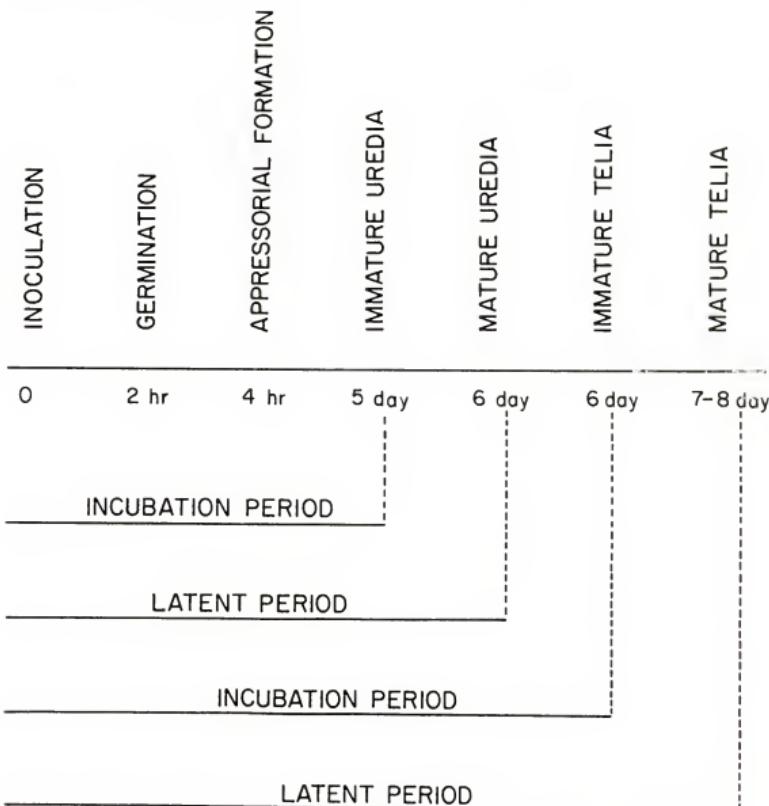
DISCUSSION

Pathogenic variability among regional and individual aecial gall isolates of C. quercuum f. sp. fusiforme is well known. Powers (1972) obtained four aeciospore collections from single galls in each of three geographic areas of the South. Infection percentages among slash pine seedlings inoculated with aeciospores ranged from 42% to 58%. Snow and Kais (1970) inoculated pine progenies with C. quercuum f. sp. fusiforme aeciospore isolates; one of the two slash pine progenies was regarded as resistant and the other was regarded as susceptible to the fusiform rust fungus. Seedlings were examined 12 months after inoculation for gall formation. The resistant slash pine progeny was moderately resistant (40-57% infection) to the North Carolina, Mississippi, and Texas isolates, but was highly susceptible to isolates from Alabama and Florida (98-100%). The susceptible slash pine progeny was highly susceptible to all geographic isolates (94-100%). Snow et al. (1976) reported that inoculum collected from a highly rust-resistant slash pine family infected more seedlings of this family than did inocula collected from the general rust population. This was interpreted as evidence of increasing pathogenic specialization in C. quercuum f. sp. fusiforme. Snow et al. (1975) inoculated slash pine families with known degrees of fusiform rust resistance using aeciospore isolates from eastern and western areas of the southeastern U.S. Variability in percent infection of two resistant and two susceptible families demonstrated pathogenic variation

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between and within the eastern and western isolates. The genetic di-
versity within populations of the host and pathogen was emphasized.

In this study, aeciospores were collected from numerous galls at
each of three locations: Macon and Valdosta, GA, and Gainesville, FL.
The collections were combined prior to mass inoculations of northern
red oak leaves, leaf discs, and water agar. No attempt was made to
determine geographical variation in pathogenicity although prior studies
had demonstrated this variation.

Dwinell (1974) surveyed 21 species of southern oak for suscepti-
bility to aeciospore inoculations of C. quercuum f. sp. fusiforme. He
reported that water and willow oaks were highly susceptible: blackjack,
cherrybark, bluejack, running, southern red, northern red, and turkey
oaks were moderately to highly susceptible; and, scarlet and black oaks
were hypersensitive to infection. In this study northern red oaks were
selected for aeciospore and urediospore inoculations due to the quali-
tative susceptibility to the fusiform rust fungus, the rapid germination
rate of the acorns, and the ample, uniform leaf surface area which
facilitated the observation of abundant development of signs and symptoms.

Utilization of the incident light microscope to assist in the ob-
servation of fungal development on unprepared leaf discs was unique to
C. quercuum f. sp. fusiforme. No staining or stripping of the leaf
surface was required for in situ examination of aeciospore and uredio-
spore germination and appressorial formation.

In studying the infection biology of C. quercuum f. sp. fusiforme
for the production of germ tubes and appressoria, several important dif-
ferences with the existing literature was observed. Walkinshaw et al.
(1967) reported primarily single germ tubes emerging from germinating

aeciospores. However, in this study the majority of germ tubes from both aeciospores and urediospores produced secondary and tertiary branching. Littlefield and Heath (1979) suggested a thigmotropic response in directional growth of the germ tubes toward stomata among urediospores of several rust fungi.   In this study a similar response could not be substantiated in scanning electron photomicrographs of germinating aeciospores and urediospores of C. quercuum f. sp. fusiforme. Aeciospore and urediospore germ tubes were not observed to form appressoria consistently at the nearest stomata; some germ tubes traversed the nearest stomata and formed appressoria upon contact with more distant stomata. Perhaps a chemical or gaseous stimulus released from stomata was responsible for the growth of the germ tubes to stomata. Failure of the spores to form appressoria over some stomata may be due to an insufficient amount of the stimulus.

Appressoria are associated with the establishment of infection by C. quercuum f. sp. fusiforme on oak and pine hosts (Eleuterius, 1968). In this study, appressoria formed only over stomata following germination of both aeciospores and urediospores and no direct penetration of leaf tissue was observed. Eleuterius (1968) reported similar results. Miller et al. (1980) reported direct penetration of slash pine needles by the germ tubes of C. quercuum f. sp. fusiforme basidiospores. In some instances, however, definite appressoria were not observed, although there were usually slight swellings of the germ tubes near the points of penetration.

The use of oak leaf discs as host substrates for aeciospore and urediospore infection had not been attempted previously. Germination

and appressorial formation were observed on leaf discs following ino-
culations with aeciospores and urediospores, but uredia or telia
were not produced. The production of uredia and telia by C. quercuum
f. sp. fusiforme on the leaf discs was not successful and was due prob-
ably to the prolonged contact of senescing leaf tissue with wet filter
paper and high relative humidity (approximately 100%) inside the petri
plates. Growth by saprophytic fungi obscured the disc surface after 2
to 4 days.

In other experiments, germination and appressorial formation fol-
lowing aeciospore and urediospore inoculations of intact northern red
oak leaves were observed and uredia and telia were produced. Intact
oak leaves were required for uredial and telial production and the ino-
culation technique using oak leaf discs was not an acceptable substi-
tute. Cronartium quercuum f. sp. fusiforme is an obligate parasite and
may be particularly sensitive to moribund host substrate. Shain and
Cornelius (1979) inoculated cottonwood (Populus deltoides Bartr.) leaf
discs with urediospores of Melampsora medusae Thuem., the causal agent
of poplar leaf rust. Optimum uredial formation was obtained when leaf
discs were washed with 52% ethanol prior to inoculation with uredio-
spores in dilute agar (0.1%) and incubated for 9 to 11 days on 1% water
agar. Less than optimal urediospore infection was attributed to an
inhibitor believed to be present on unwashed leaf surfaces. Perhaps a
similar inhibitory mechanism for infection was responsible for the fail-
ure of uredia and telia to form on northern red oak leaf discs in this
study.

Siggers (1947) reported that the cardinal temperature points for
aeciospore germination on water agar were 11 and 29 C with the optimum

temperature for germination at 21 C. The temperature limits from water agar experiments in this study were similar to those found by Siggers. The maximum percentage of aeciospores germinated between 15 and 21 C; germination at other temperatures was reduced. This suggests an important ecological and temporal relationship may exist between germination and appressorial formation. Optimum aeciospore dispersal occurs in the spring in the early afternoon when temperature and wind velocity are high (Webb and Schmidt, 1981). If germination began shortly after deposition, ambient temperatures would still be as high as approximately 21 C. Optimum formation of appressoria several hours later would coincide with cooler temperatures (as low as 15 C) of the later afternoon and early evening. The theoretical maxima for percentage aeciospore germination on water agar at 15, 20, and 21 C were 60, 70, and 75%, respectively. At temperatures above and below this range, i.e., 10, 25, 27, and 30 C, the theoretical maxima were approximately 10, 25, 3, and 1%, respectively. Germination at the lowest temperature required 2.3 and 4.3 hours to reach 10 and 90%, respectively, of the theoretical maximum. Germination at the optimum required 1.3 and 4.9 hours to reach 10 and 90%, respectively, of the theoretical maximum. At 30 C, the probable influence of heat upon increased spore drying and respiration resulted in a low theoretical germination which occurred quickly, i.e. 1 hour to 90% of the theoretical maximum germination.

Compared to water agar inoculation, the theoretical maximum percentage germination of aeciospores on leaf discs was greatly reduced; for example, approximately 78% germinated on water agar but only 34% germinated on leaf discs at the optimum temperature of 21 C. Also, the

range of germination percentages was broader around the optimum (15–21 C) on water agar than on leaf discs at 21 C. Addition of the oak host component differed from Siggers' (1947) method; the optimum temperature for germination was substantiated, but the percentage of the aecio-spores germinating at each of the seven temperature levels was lower. The times required to reach 90% theoretical maximum germination were approximately twice as long as those for spores on water agar.

Siggers (1947) reported that the lower and upper temperature limits for urediospore germination on water agar were 8 and 29 C, respectively, with 18 C as the optimum temperature. A slightly different range of thermal limits, 10 and 30 C, respectively, was observed in this experi-ment on water agar. The theoretical maximum percentage germination of urediospores on water agar was uniformly high (50–75%) except at 30 C when only 4% of the urediospores germinated. The times to 10 and 90% of the theoretical maximum were approximately equivalent except at 30 C where the former required the least four times as long and the latter twice as long compared to lower temperatures. As with the aeciospore germination experiments using leaf discs, urediospore germination was higher (55 to 74%) across a more narrow temperature range (15 to 21 C) than that in water agar trials. The times to 10 and 90% theoretical maximum germination of urediospores on leaf discs were approximately the same as in the aeciospore-leaf disc experiments in that much longer time periods were required.

Walkinshaw et al. (1967) reported that the germination of C. quercuum f. sp. fusiforme aeciospores was dependent upon imbibition of water. In this study, maximum aeciospore germination and appressorial

formation were dependent upon a total of at least 6 hours of leaf surface moisture. Six hours were required for 90% of the maximum average observed aeciospore germination (48%) while 8 hours were required for 90% of the maximum average appressorial formation (38.0%). The 2-hour difference between germination and appressorial production was presumed due to the formation of the appressorial structure. Interruptions in the moisture period had no significant effect on the eventual numbers of aeciospores that germinated and formed appressoria, but did increase the total time necessary for both processes. According to Walkinshaw et al. (1967), energy for aeciospore germination is provided by lipid utilization. Interruption of the moisture period, and therefore the lipid utilization process, may have delayed eventual germination and appressorial formation, but apparently did not permanently retard the processes.

Maximum urediospore germination required at least a total of 3 hours of leaf surface moisture as compared to 6 hours necessary for aeciospore germination. Maximum urediospore appressorial formation required at least a total of 4 hours of leaf surface moisture as compared to 6 hours needed for aeciospore appressorial formation. A positive relationship existed between longer periods of moisture and higher percentage urediospore appressorial formation. One hour after the initiation of germination and appressorial formation, an interruption of leaf surface moisture for either 1 or 3 hours did not result in a significant reduction of either process. Studies encompassing longer moisture interruption periods are necessary before the effects upon germination and appressorial formation are fully delineated.

Snow and Roncadori (1965) reported that with increasing age, water oak leaves become increasingly resistant to both aeciospore and urediospore infection. In this study with detached 2- to 20-day-old leaf discs, percentages of aeciospore and urediospore germination and appressorial formation were only slightly different among the different age classes. The failure of uredia and telia to develop on older leaves probably occurred during some phase of colonization of C. quercuum f. sp. fusiforme.

Previous studies outlined temperature and moisture conditions for aeciospore and urediospore germination (Snow and Roncadori, 1965 and Roncadori and Matthews, 1966). In this study the non-linear regression analyses of temperature and moisture optima for aeciospore and urediospore germination and appressorial formation were unique to C. quercuum f. sp. fusiforme and provided predictive utility for the performance of the pathogen under laboratory conditions. This analysis significantly enhanced the predictability of percentages of germination and appressorial formation at various temperature and moisture conditions. Aeciospore and urediospore germination and appressorial formation in the temperature and leaf age experiments were modelled using logistic equations since normal curves could be representative of the data. Goodness of fit using the logistic equations was apparent from the high R^2 values. However, other models may describe the curves more accurately. Waggoner and Parlange (1974) and Schröedter (1975) have analyzed spore development under varying temperatures and concluded that a normal curve or a polynomial curve, respectively, were acceptable representatives of spore development.

The rates of and times to 10 and 90% of the theoretical maximum germination and appressorial formation further clarified the temporal behavior of the pathogen. Further analysis should be conducted to predict germination and appressorial formation under fluctuating temperature and moisture conditions in the field.

In studying the temporal sequence of uredial and telial formation, Hedgcock and Siggers (1949) reported the appearance of uredia 9 to 11 days after inoculation with aeciospores. Snow and Roncadori (1965) observed initial uredia formation 5 to 6 days after aeciospore inoculation, and telia appeared after 7 to 8 days. Siggers (1947) first observed telia 6 days after inoculation with aeciospores. In this study uredia were first observed 6 days after inoculation with aeciospores. A range of .08 to .42 uredial sori/cm^2 of leaf surface existed throughout the subsequent 10 day period. The maximum number of uredia formed on leaves 5-days-old at the time of inoculation. The maximum infectious period for the population of uredial sori (which was the total time that the sori were infectious) was 9 days following aeciospore inoculation of 2-day-old leaves.

Telia were observed after 8 days after aeciospore inoculations with a range of .26 to 2.48/cm^2 of leaf surface existing throughout the remaining 14 days of the experiments. The maximum number of telia formed on leaves 6-days-old at the time of inoculation. The latent period for telia was approximately 9 days based on the time from inoculation with aeciospores to the development of probasidia on the telial column (which were observed during scanning electron microscopic observations on infection biology). The number of viable uredia decreased after 8

to 10 days. However, the number of telial columns present remained constant to the end of the experiment or 22 days after inoculation. No uredia developed on leaves 8 days or older and telia did not develop on leaves 16 days or older. The average population ratios of telia to uredia for 2-, 5-, and 6-day-old leaves following aeciospore inoculation were 1.53, 2.00, and 31.00, respectively. As leaf age increased the average numbers of uredia decreased while the average numbers of telia remained relatively constant.

Secondary infection of oak leaves by urediospores apparently did not occur since the number of telia remained relatively constant per leaf age class beginning 15 days and 14 days after aeciospore and urediospore inoculations, respectively. Secondary urediospore infection apparently did not occur prior to these periods since the number of telia increased linearly rather than geometrically. If secondary infection cycles had occurred the number of telia would have been expected to continue increasing rather than remain constant. This may have been due to host resistance which may have increased as the oak leaves matured. Also, since there was no rewetting of the leaf surface after the initial inoculation, moisture conditions which facilitated the initial urediospore infection would not have been present for secondary infections.

Czabator (1971) reported that aeciospore inoculations of oak leaves resulted more frequently in the formation of telia than uredia. Furthermore, the uredial stage, presumably necessary for local inoculum buildup, may be bypassed when unfavorable host conditions prevailed. Fewer uredia and telia formed on oak leaves as the ages of the leaves at the time of inoculation increased. Leaves 8 days or older when inoculated

with aeciospores did not exhibit uredia; leaves 11 days or older when inoculated with urediospores did not exhibit uredia. The maximum number of uredia and telia occurred after approximately 8 days and 14 days, respectively, after inoculation with either aeciospores or urediospores. More telia were observed than uredia following aeciospore inoculation and the occurrence of a telium was not necessarily preceeded by formation of a uredium. Telia, but no uredia, were produced on leaves older than 6 days and 9 days at the time of aeciospore and urediospore inoculations, respectively. The occurrence of unfavorable host conditions, such as leaf age, which led to bypassing uredial formation was evident and warranted further investigation.

Jewell and Eleuterius (1963) reported amphiphyllous uredia and telia but these occurred simultaneously from the same mycelial mass. In this study hyperphyllous uredia and telia occurred without accompanying hypophyllous uredia and telia.

Snow and Roncadori (1965) inoculated susceptible oak leaves with urediospores and observed uredial formation but did not report the time period for initial occurrence. In this study uredia first appeared 5 days after inoculation with urediospores. A range of .04 to 1.5 uredial sori/cm^2 of leaf surface existed throughout the 25 days of the experimental period. The maximum number of uredia and telia formed when leaves were 2- and 7-days-old, respectively, at the time of inoculation with urediospores. The maximum infectious period for the population of uredial sori was at least 24 days (which was the termination of the experiment) following urediospore inoculation of 2-, 5-, 7-, and 9-day-old leaves. The average number of uredial sori decreased approximately

9 days after inoculation. No uredia developed on leaves 11 days or
older.

Czabator (1971) reported that telia first appeared about 8 days
after inoculation with urediospores. In this study telia were first
observed after 6 days with a range of .67 to 4.4/cm^2 of leaf surface
existing throughout the subsequent 24-day period. The number of viable
telial columns remained constant after 14 days of the 30-day experimen-
tal period. No telia developed on leaves inoculated when 14 days old
or older. The average population ratios of telia to uredia for 2-, 5-,
7-, and 9-day-old leaves following urediospore inoculation were 1.20,
3.82, 73.83, and 107.50, respectively. As leaf age increased the average
numbers of uredia decreased while the average numbers of telia remained
relatively constant.

Vanderplank (1963) stated that no increase in disease can begin
unless conditions of the host, pathogen, and environment interact such
that:

$$iR_c \geq 1$$

where,   i = infectious period (time, usually days) over which a
             lesion remains infectious,

R_c = correct basic infection rate (corrected for removals);
        it is expressed as a rate, i.e., per unit per day.

The infectious period for an individual pustule was not determined but
the maximum infectious period for the population of pustules was 9 days
and at least 24 days with aeciospore and urediospore inoculations,
respectively. The inoculation of oak leaves with 1000 $\pm$ 100 aecio-
spores/cm^2 of leaf surface resulted in the formation of 0.22 uredial
sorus and 1.07 telial columns. Approximately 5000 and 1000 aeciospores
were necessary to form 1 uredial sorus and 1 telial column, respectively.

Inoculation with 1000 ± 100 urediospores per cm^2 of leaf surface resulted in the production of 0.66 uredia and 2.63 telia. Approximately 1500 and 377 urediospores were necessary to form 1 uredial sorus and 1 telial column, respectively. To maintain at least a constant level of urediospore infection, each uredial pustule must produce at least 1500 urediospores during the infectious period of that sorus. Conversely, the prerequisite of 1500 urediospores to produce 1 effective uredial sorus, as determined in this study, may not be realistic. Perhaps fewer spores are needed in order to perpetuate the fungus.

Despite the relatively high concentrations of aeciospores and urediospores (1000 ± 100 spores/cm^2 of leaf surface), few telia and even fewer uredia were formed. Mehta and Zadoks (1970) investigated urediospore production of P. recondita f. sp. triticina Rob. ex Desm. on wheat. Three concentrations of urediospores (14,633, 3565, and 1784 spores/leaf) yielded 1917, 920, and 305 uredial pustules per leaf, respectively, and the ratios of pustules/leaf to urediospores/leaf were 0.13, 0.26, and 0.17, respectively. These ratios were considerably higher than the ratios for uredial and telial formation on oak leaves from aeciospore inoculations, 0.00022 and 0.00107, respectively, and urediospore inoculations, 0.00066 and 0.00263, respectively. The concentrations of aeciospores and urediospores were believed to be realistic.

Schmidt and Webb (unpublished data) calculated aecial aeciospore concentrations during the peak of several spring sporulation seasons of over 1600 spores/cm^2 of trapping surface from a spore trap located 10 m from a slash pine plantation infected with C. quercuum f. sp. fusiforme. The concentration of 1000 spores/cm^2 of leaf surface in this study may

have represented inoculum deposition on leaves near severely infected pines. Since only one point source on the infinite inoculum curve was studied, perhaps additional spore concentrations could have provided different inoculum efficiency schedules.

Dwinell (1974) surveyed the relative susceptibility of 21 southern oak species to mass inoculations of C. quercuum f. sp. fusiforme aeciospores and the potential for telial production on each host species. Six to seven days after cessation of uredial development, the numbers of telia/cm^2 of oak leaf surface were counted and were the basis for assigning relative host susceptibility and telium productivity. All the oaks tested were susceptible based upon the production of telia, but inoculum efficiency can not be calculated since the initial concentration of the aeciospore inoculum was not specified.

Effects of weather upon C. quercuum f. sp. fusiforme basidiospore infection have been thoroughly examined (Davis and Snow, 1968; Kais, 1963; Powers and Roncadori, 1966; Snow and Froelich, 1968; and Snow et al., 1968). Similar investigations of weather effects on aeciospore infection are lacking. Siggers (1949) reported that warm weather in late winter and early spring coupled with typical ample rainfall stimulated C. quercuum f. sp. fusiforme aeciospore production from infected pines. Oak infection developed rapidly when average temperature exceeded 15 C. Roncadori and Matthews (1966) observed significantly greater germination of stored aeciospores when temperatures ranged between 15 and 21 C. In this study, optimum aeciospore germination and appressorial formation also occurred at 21 and 15 C, respectively, and optimum urediospore germination and appressorial formation occurred at 18 C.

The potential for aeciospore infection of oaks is increased when warm humid weather is initiated each spring.

Adaptation of the pathogen to these temperature and relative humidity optima is an evolutionary process whereby aeciospore release from colonized pines coincides with bud break and early leaf flush on oaks. Oak leaves 4 to 6 days old or older exhibited decreased uredial formation following inoculation with aeciospores (Snow and Roncadori, 1965). In this study with oak leaf discs, percentage of infection was similar among 2- to 11-day-old leaves which emphasized the role of oak leaf resistance. Despite the occurrence of optimum weather conditions for infection over a long period, i.e., March through early June, the maximum potential for infection of individual leaves is reduced since oak leaves are susceptible for only brief periods. Kranz (1974) reported a similar pattern for $E.$ $graminis$. Due to the consistent production of leaves during the growth period, leaf tissue susceptible for aeciospore infection is constantly available. Despite the magnitude of present levels of infection, higher incidence would probably result if emerging leaves were susceptible for even slightly longer periods. This may help to explain the large regional volume of aeciospores necessary to perpetuate yearly infection in the southeastern U.S.

Zadoks and Schein (1979) stated that those airborne spores effective in establishing infection were only a small fraction of the total output. Furthermore, the minimum effective rate which perpetuates infection and subsequent colonization is proportional to the total spore output. The inoculum efficiency schedules for aeciospore and uredio-spore infection of oak seedlings underscored the ability of the pathogen

to produce abundant levels of sporidial inocula. Initial concentrations of 1000 ± 100 spores/cm^2 of oak leaf surface resulted in about 1 and 3 telia, respectively, from aeciospore and urediospore inoculations. Snow (unpublished data) hypothesized that under optimum temperature and humidity conditions, about 1000 basidiospores could be produced daily per linear mm of telial column. Since telial columns are usually at least several mm long and are often abundant on infected oak leaves, the potential for increased sporidial production is enhanced many times. Thus, large numbers of aeciospores and urediospores are necessary to produce a single telium but each telium may produce many thousands of basidiospores. Snow (personal communication) obtained infection of slash pine seedlings under optimum laboratory conditions by placing as few as 30 to 60 basidiospores on a single pine needle. Squillace and Wilhite (1977) and Hollis and Schmidt (1977) demonstrated the positive correlation of fusiform rust incidence with local susceptible oak abundance. Rapid increases in fusiform rust incidence since the early 1950's is due partly to the productivity of inoculum on oaks (Dinus, 1974). However, further clarification of these inoculum efficiency schedules under field conditions is necessary to substantiate these hypotheses.

CONCLUSIONS

Aeciospore and urediospore germination occurred about 15 minutes
after inoculation onto oak leaf discs or water agar. Both spore types
produced branched and unbranched germ tubes. Growth of these germ tubes
towards stomata appeared random. Appressoria developed only over
stomata; penetration was observed to occur only through stomata.

The optimum temperatures of aeciospore and urediospore germination
on water agar and oak leaf discs were 21 and 18 C, respectively. Maxi-
mum percentage germination of aeciospores on leaf discs was much less
and occurred over a more narrow range of optimum temperatures than in
the water agar trials.

The optimum temperatures for aeciospore and urediospore appressorial
formation were 15 and 18 C, respectively. Aeciospores required
longer periods to reach 10 and 90% of the theoretical maximum appressor-
ial formation than did urediospores. At higher temperatures
percentage aeciospore appressorial formation was progressively reduced
whereas percentage urediospore appressorial formation was approximately
constant.

At least 6 hours of leaf surface moisture were required for abun-
dant germination by aeciospores. No significant differences in per-
centage germination occurred between 6 and 9 hours of moisture. Per-
centage appressorial formation increased linearly as the moisture dura-
tion increased. Urediospore germination required more than 2 hours of

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surface moisture. Percentage appressorial formation steadily increased and was maximized after 24 hours.

Aeciospore and urediospore germination and appressorial formation were slightly greater when the moisture period was not interrupted. The availability of leaf surface moisture was important in perpetuating germination and appressorial formation.

Percentages of aeciospore and urediospore germination and appressorial formation were approximately constant over all leaf disc age classes although urediospore values were slightly higher.

In vitro sporulation by C. quercuum f. sp. fusiforme did not occur on oak leaf discs following aeciospore or urediospore inoculations.

Uredial sori developed 6 days after aeciospore inoculation of 2- to 6-day-old leaves. The maximum number of uredia formed on leaves 5-days-old at the time of inoculation. No uredial sori developed on 8-, 11-, or 16-day-old leaves. The latent period for uredial sori was approximately 7 days; the infectious period for the population of uredial sori was approximately 9 days on leaves 2-days-old at time of inoculation.

The latent period for telia was 8 to 9 days after aeciospore inoculation of 5-, 6-, and 11-day-old leaves. Telial columns appeared after 15 days when 2- and 8-day-old leaves were inoculated. The maximum number of telia formed on leaves 6-days-old at the time of inoculation. Telia, but not uredia, were produced on leaves older than 6 days and 9 days at the time of aeciospore and urediospore inoculations, respectively.

The average population ratios of telia to uredia for 2-, 5-, and 6-day-old leaves following aeciospore inoculation were 1.53, 2.00, and

31.00, respectively. The average population ratios of telia to uredia for 2-, 5-, 7-, and 9-day-old leaves following urediospore inoculation were 1.20, 3.82, 73.83, and 107.50, respectively.

Inoculation with 1000 ± 100 aeciospores/cm^2 of oak leaf surface resulted in approximately .22 uredial pustule/cm^2 after 6 to 16 days and 1.07 telial columns/cm^2 after 8 to 20 days on 2- to 6-day-old leaves and 2- to 11-day-old leaves, respectively.

Uredial sori developed 5 days after urediospore inoculation of 2- to 9-day-old leaves. The maximum number of uredia and telia formed when leaves were 2- and 7-days-old, respectively, at the time of inoculation with urediospores. The latent period for uredial development was approximately 6 days; the infectious period for the population of uredial sori was at least 24 days (which was the termination of the experiment) when leaves were 2-, 5-, 7-, and 9-days-old at the time of inoculation with urediospores.

Telial columns appeared 6 days after urediospore inoculation of 5- to 11-day-old leaves and the latent period was approximately 7 to 8 days.

Inoculation of 1000 ± 100 urediospores/cm^2 of oak leaf surface resulted in approximately .66 uredial pustule/cm^2 after 5 to 30 days and 2.63 telial columns/cm^2 after 6 to 30 days on 2- to 9-day-old leaves and 2- to 11-day-old leaves, respectively.

FUTURE RESEARCH

The effect of oak genotypic resistance upon aeciospore and urediospore germination and appressorial formation should be investigated further. The identification of susceptible, resistant, and immune oak species to aeciospore and urediospore inoculation should be more thoroughly investigated. Temperature and moisture effects on aeciospore and urediospore germination and appressorial formation and uredial and telial formation may then be conducted in the field to clarify optimum conditions across a range of interspecific and intraspecific oak genotypes. Inoculation with a range of fungal genotypes of C. quercuum f. sp. fusiforme would be performed to more thoroughly examine pathogenic variability relative to various temperature and moisture conditions.

Additional research should be conducted on the effects of interruption of moisture periods on aeciospore and urediospore germination and appressorial formation. A sufficiently large range of interruption periods was not incorporated into this study.

The importance of secondary infection of oak leaves by urediospores warrants further research. Continued laboratory and field investigations of the effects of temperature, leaf surface moisture, and light quality and quantity upon the quantity of urediospores produced are needed. In addition, the phenomenon of the bypassing of the uredial stage needs to be examined. The ratio of the production of uredial to

telial sori needs to be studied in order to ascertain the importance of possible host resistance to the production of the structures.

Inoculum efficiency schedules for aeciospore and urediospore germination and appressorial formation need to be refined. More quantitative and comprehensive experiments utilizing a broad range of environmental conditions and spore concentrations are necessary. Also, a range of fungal isolates with varying pathogenicity should be incorporated into inoculation using a range of oak genotypes.

LITERATURE CITED

Allen, R. F. 1928. A cytological study of Puccinia glumarum on Bromus marginatus and Triticum vulgare. J. Agric. Res. 36:487-513.

Anon. 1977. Influence of climate on fusiform rust incidence and distribution. Page 80 in Management of fusiform rust in southern pines. R. J. Dinus and R. A. Schmidt, eds. Symp. Proc. Univ. Fla., Gainesville. 162 p.

Benny, G. L. and H. C. Aldrich. 1975. Ultrastructure observations on septal and merosporangial ontogeny in Linderina pennispora. Can. J. Bot. 53:2325-2326.

Berger, R. D. 1976. Computer simulation of Cercospora apii and Helminthosporium turcicum. Proc. Amer. Phytopathol. Soc. (Abstr.) 3:217.

Boyce, J. S. 1961. Forest pathology. McGraw-Hill, New York. 572 p.

Burdsall, H. H. and G. A. Snow. 1977. Taxonomy of Cronartium quercuum and Cronartium fusiforme. Mycologia 69:503-508.

Colley, R. H. 1918. Parasitism, morphology, and cytology of Cronartium ribicola. J. Agric. Res. 15:619-660.

Czabator, F. J. 1971. Fusiform rust of southern pines - a critical review. USDA For. Serv. Res. Pap. SO-65. 39 p.

Davis, R. T. and G. A. Snow. 1968. Weather systems related to fusiform rust infection. Plant Dis. Reptr. 52:419-422.

Dinus, R. J. 1974. Knowledge about natural ecosystems as a guide to disease control in managed forests. Proc. Am. Phytopathol. Soc. 1:184-190.

Dinus, R. J. and R. A. Schmidt, eds. 1977. Management of fusiform rust in southern pines. Symp. Proc. Univ. Fla., Gainesville. 163 p.

Dwinell, L. D. 1969. Volume-size distribution of aeciospores of Cronartium fusiforme and C. quercuum. Phytopathology 59:1024-1025.

Dwinell, L. D. 1974. Susceptibility of southern oaks to Cronartium fusiforme and Cronartium quercuum. Phytopathology 64:400-403.

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Eleuterius, L. N. 1968. Morphology of Cronartium fusiforme in Quercus nigra. Phytopathology 58:1487–1492.

Goddard, R. E. and O. O. Wells. 1977. Susceptibility of southern pines to fusiform rust. Pages 52–58 in Management of fusiform rust in southern pines. R. J. Dinus and R. A. Schmidt, eds. Symp. Proc. Univ. Fla., Gainesville. 162 p.

Gooding, G. V., Jr. and H. R. Powers, Jr. 1965. Serological comparison of Cronartium fusiforme, C. quercuum, and C. ribicola by immunodiffusion tests. Phytopathology 55:670–674.

Griggs, M. M. and R. A. Schmidt. 1977. Increase and spread of fusiform rust. Pages 32–38 in Management of fusiform rust in southern pines. R. J. Dinus and R. A. Schmidt, eds. Symp. Proc. Univ. Fla., Gainesville. 162 p.

Harder, D. E. 1976. Electron microscopy of urediospore formation in Puccinia coronata avenae and P. graminis avenae. Can. J. Bot. 54: 1010–1019.

Hedgcock, G. G. and P. V. Siggers. 1949. A comparison of the pine-oak rusts. USDA Tech. Bull. No. 978. 30 p.

Hiratsuka, Y. 1973. The nuclear cycle and the terminology of spore states in Uredinales. Mycologia 65:432–443.

Hobbs, E. L. 1962. Some factors affecting germinability of urediospores of Puccinia coronata. Phytopathology 52:1223–1225.

Hollis, C. A. and R. A. Schmidt. 1977. Site factors related to fusiform rust incidence in north Florida slash pine plantations. For. Sci. 23:69–77.

Hughes, S. J. 1970. Ontogeny of spore forms in Uredinales. Can. J. Bot. 48:2147–2157.

Jewell, F. F. and L. N. Eleuterius. 1963. Amphiphyllous uredia and telia of southern fusiform rust. Plant Dis. Rep. 47:65.

Jewell, F. F., R. P. True, and S. L. Mallett. 1962. Histology of Cronartium fusiforme in slash pine seedlings. Phytopathology 52: 850–858.

Johnson, T. 1934. A tropic response in germ tubes of urediospores of Puccinia graninis tritici. Phytopathology 24:80–82.

Kais, A. G. 1963. In vitro sporidial germination of Cronartium fusiforme. Phytopathology 53:987–988.

Kais, A. G. and G. A. Snow. 1972. Host response to various isolates of Cronartium quercuum and Cronartium fusiforme. Pages 495–503 in Biology of rust resistance in forest trees. USDA Misc. Publ. 1221. 681 p.

Kato, H. 1974. Epidemiology of rice blast disease. Rev. Plant Protec. Res. 7:1-20.

Kranz, J. 1974. The role and scope of mathematical analysis and modeling in epidemiology. Pages 1-54 in Epidemics of plant diseases. Springer-Verlag, Berlin. 170 p.

Lewis, B. G. and J. R. Day. 1972. Behavior of urediospore germ tubes of Puccinia graminis tritici in relation to the fine structure of wheat of leaf surfaces. Trans. Br. Mycol. Soc. 58:139-145.

Littlefield, L. J. and M. C. Heath. 1979. Ultrastructure of rust fungi. Academic Press, New York. 277 p.

Maheshwari, R. and A. C. Hildebrandt. 1967. Directional growth of the urediospore germ tubes and stomatal penetration. Nature 214: 1145-1146.

Mehta, Y. R. and J. C. Zadoks. 1970. Urediospore production and sporulation period of Puccinia recondita f. sp. triticina on primary leaves of wheat. Neth. J. Pl. Path. 76:267-276.

Miller, T. 1970. Inoculation of slash pine seedlings with stored basidiospores of Cronartium fusiforme. Phytopathology 60:1773-1774.

Miller, T., E. B. Cowling, H. R. Powers, Jr., and T. W. Blalock. 1976. Types of resistance and compatibility in slash pine seedlings infected by Cronartium fusiforme. Phytopathology 66:1229-1235.

Miller, T., R. F. Patton, and H. R. Powers, Jr. 1980. Mode of infection and early colonization of slash pine seedlings by Cronartium quercuum f. sp. fusiforme. Phytopathology 70:1206-1208.

Patton, R. F. and D. W. Johnson. 1966. Penetration of pine needles by Cronartium basidiospores labeled with a fluorescent brightener. (Abstr.) Phytopathology 56:894.

Phelps, W. R. 1977. Incidence and distribution of fusiform rust. Pages 25-31 in Management of fusiform rust in southern pines. R. J. Dinus and R. A. Schmidt, eds. Symp. Proc. Univ. Fla., Gainesville. 163 p.

Phelps, W. R. and C. W. Chellman. 1975. Impact of fusiform rust in north Florida slash pine plantations. Plant Dis. Rep. 59:781-785.

Politowski, K. and J. A. Browning. 1974. The effect of temperature, light and dew duration on the development of infection structures of Puccinia coronata avenae. (Abstr.). Annu. Proc. Am. Phytopathol. Soc. 1:107.

Politowski, K. and J. A. Browning, 1975. Effect of temperature, light and dew duration on relative numbers of infection structures of Puccinia coronata avaea. Phytopathology 65:1400-1404.

Powers, H. R., Jr. 1972. Testing for pathogenic variability with Cronartium fusiforme and C. quercuum. Pages 505-511 in Biology of rust resistance in forest trees. USDA Misc. Publ. 1221. 681 p.

Powers, H. R., Jr. 1975. Relative susceptibility of five southern pines to Cronartium fusiforme. Plant Dis. Rep. 59:312-314.

Powers, H. R., Jr., J. P. McClure, H. A. Knight, and G. F. Dutrow. 1974. Incidence and financial impact of fusiform rust in the south. J. For. 72:398-401.

Powers, H. R., Jr. and R. W. Roncadori. 1966. Teliospore germination and sporidial production by Cronartium fusiforme. Plant Dis. Reptr. 50:432-434.

Roncadori, R. W. and F. R. Matthews. 1966. Storage and germination of aeciospores of Cronartium fusiforme. Phytopathology 56:1328-1329.

Schmidt, R. A., R. E. Goddard, and C. A. Hollis. 1974. Incidence and distribution of fusiform rust in slash pine plantations in Florida and Georgia. Fla. Agric. Exp. Stn. Tech. Bull. 763, 21 p.

Schroedter, H. 1965. Methodisches zur Bearbeitung phytometeoropathologischer Untersuchungen, dargestellt am Beispiel der Temperaturrelation. Phytopathol. Z. 53:154-166.

Shain, L. and P. L. Cornelius. 1979. Quantitative inoculation of eastern cottonwood leaf tissue with Melampsora medusae under controlled conditions. Phytopathology 69:301-304.

Siggers, P. V. 1947. Temperature requirements for generation of spores of Cronartium fusiforme. Phytopathology 37:855-864.

Siggers, P. V. 1949. Weather and outbreaks of the fusiform rust of southern pines. J. For. 47:802-806.

Snow, G. A., R. J. Dinus, and A. G. Kais. 1975. Variation of pathogenicity of diverse sources of Cronartium fusiforme on selected slash pine families. Phytopathology 65:170-175.

Snow, G. A., R. J. Dinus, and C. H. Walkinshaw. 1976. Increase in virulence of Cronartium fusiforme on resistant slash pine. Phytopathology 66:511-513.

Snow, G. A. and R. C. Froelich. 1968. Daily and seasonal dispersal of basidiospores of Cronartium fusiforme. Phytopathology 58:1532-1536.

Snow, G. A., R. C. Froelich, and T. W. Popham. 1968. Weather conditions determining infection of slash pine by Cronartium fusiforme. Phytopathology 58:1537-1540.

Snow, G. A. and A. G. Kais. 1970. Pathogenic variability in isolates of _Cronartium fusiforme_ from five southern states. Phytopathology 60:1730-1731.

Snow, G. A. and R. W. Roncadori. 1965. Oak leaf age and susceptibility to _Cronartium fusiforme_. Plant Dis. Rep. 49:972-973.

Squillace, A. E. and L. P. Wilhite. 1977. Influence of oak abundance and distribution on fusiform rust. Pages 59-70 in R. J. Dinus and R. A. Schmidt eds. Management of fusiform rust in southern pines. Symp. Proc. Univ. Fla., Gainesville. 163 p.

Teng, P. S. and J. C. Zadoks. 1980. Computer simulation of plant disease epidemics. Pages 22-31 in McGraw-Hill Yearbook of Science and Tech., New York. 447 p.

Van Arsdel, E. P., A. J. Riker, and R. F. Patton. 1956. The effects of temperature and moisture on the spread of white pine blister rust. Phytopathology 46:307-318.

Vanderplank, J. E. 1963. Plant diseases: epidemics and control. Academic Press, New York. 349 p.

Vanderplank, J. E. 1975. Principles of plant infection. Academic Press, New York. 216 p.

Vargas, J. M., Jr., H. C. Young, Jr., and E. E. Saari. 1967. Effect of light and temperature on urediospore germination, infection, and disease development of _Puccinia cynodontis_, and isolation of pathogenic races. Phytopathology 57:405-409.

Waggoner, P. E. and J. G. Horsfall. 1969. EPIDEM- a simulation of plant disease written for a computer. Conn. Agr. Exp. Sta. Bull. No. 698. 80 p.

Waggoner, P. E. and J. Y. Parlange. 1974. Mathematical model for spore germination at changing temperature. Phytopathology 64: 605-610.

Waggoner, P. E. and J. Y. Parlange. 1974. Verification of a model of spore germination at variable, moderate temperatures. Phytopathology 64:1192-1196.

Walker, J. C. 1957. Plant pathology. McGraw-Hill Book Co., Inc., New York. 707 p.

Walkinshaw, C. H., J. M. Hyde, and J. VanZandt. 1967. Fine structure of quiescent and germinating aeciospores of _Cronartium fusiforme_. J. Bacteriol. 94:245-254.

Webb, R. S. and R. A. Schmidt. 1981. The diurnal periodicity of
  <u>Cronartium quercuum</u> f. sp. <u>fusiforme</u> aeciospore dispersal in
  north Florida and associated meteorological variables. (Abstr.)
  Southwide Forest Disease Workshop. University of Florida,
  Gainesville. Fl.

Wynn, W. K. 1976. Appressorium formation over stomates by the bean
  rust fungus: Response to a surface contact stimulus. Phyto-
  pathology 66:136-146.

Zadoks, J. C. 1965. Epidemiology of wheat rusts in Europe. FAO
  Plant Prot. Bull. 13:97-108.

Zadoks, J. C. 1978. Methodology of epidemiological research. Pages
  63-94 <u>in</u> Plant disease an advanced treatise, Vol. II. J. G.
  Horsfall and E. B. Cowling, eds. Academic Press, New York.
  435 p.

Zadoks, F. C. and R. D. Schein. 1979. Epidemiology and plant disease
  management. Oxford University Press, New York. 427 p.

BIOGRAPHICAL SKETCH

Patricia Gardner Patterson was born in Ocala, Florida, on June 10, 1949. She attended Williston schools and graduated from Williston High School in June, 1967. She enrolled in the University of Florida, Gainesville, Florida, in September, 1967, and graduated in June, 1971, with a B.S. in biology-chemistry education. During her undergraduate years she was a member of the President's List for academic achievement and Phi Theta Kappa, a national honor society.

She was employed as a chemist by Ordnance Research Institute in the summer of 1971 and was employed as a chemistry-physiology instructor at Niceville High School from August, 1971, until June, 1979. In June, 1973, she entered graduate school in the College of Education at the University of Florida and graduated in June, 1974, with a M.S. in biology education. In June, 1979, she entered the University of Florida as a graduate research assistant in the Department of Plant Pathology to pursue a curriculum culminating in the Doctor of Philosophy degree in plant pathology.

She has two children, LaBeth and Amber.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert A. Schmidt, Chairman
Professor of Plant Pathology and
Forest Resources and Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.
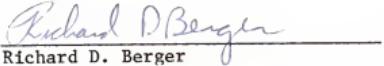
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George M. Blakeslee
Assistant Professor of
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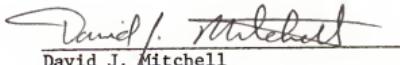
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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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